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Field and laboratory studies were conducted to characterize the chemical properties of municipal sewage sludges, to evaluate the fate of sludge components in soils, and to determine the distribution of trace metals in milling fractions of grains grown on sludge-treated soils.

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimonies to the deterioration of our natural environment. The complexity of that environment and the interplay of its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution; it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems to prevent, treat, and manage wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, to preserve and treat public driking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research and provides a most vital communications link between the researcher and the user community.

This report summarizes the results obtained from a series of field and laboratory studies designed to evaluate the chemical and microbial processes occurring in soils amended with stabilized municipal sewage sludges. The studies conducted involved characterization of sludge chemical properties, transformations of sludge components in soils treated with sludges and distribution of trace metals in milling fractions of grains grown on soils amended with sludge.

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ABSTRACT

A series of field and laboratory studies were conducted to determine the chemical and microbial processes occurring in soils following application of anaerobically digested municipal sewage sludge. Characterization of the organic components in sewage sludges indicated that fatty acid esters were a predominant form present. Carbohydrate type materials accounted for < 2.55% of the sludge, while amino acids accounted for the majority of organic nitrogen. The ability of the municipal sludges to retain added heavy metals was evaluated by adsorption experiments. Sludges possessed the capacity to retain appreciable amounts of cadmium, copper, nickel and zinc. The primary mechanisms involved in retention of added metal salts by the calcareous sludges studied were likely precipitation as carbonates and sorption by organic matter and hydrous oxides. A heavy liquid extraction procedure was developed to separate sewage sludges into organic and inorganic fractions. This approach indicated that the majority of metals naturally present in the sludge were associated with organic components.

Studies were conducted to evaluate the spectroscopic properties of sludge organic matter. Humic acids extracted from soil, sludge, and sludge-amended soil indicated that the sludge humic acid was more aliphatic and contained decreased amounts of carboxyl and phenolic hydroxyl functional groups when compared to soil humic acids. Proteinaceous materials were associated with the sludge humic acid and infrared investigations indicated that the amide group in residual proteinaceous materials could be involved in the retention of heavy metals such as copper. Electron spin resonance studies indicated that copper(II) was bound to oxygen containing donor ligands in the humic acid fraction of sewage sludge. Theoretical studies were conducted to evaluate the effects of pH and redox on the behavior of five synthetic chelating agents in soil systems. The DTPA soil test procedure appears to have a sound theoretical basis for evaluating plant available metals in soils amended with sludges.

Laboratory incubation experiments were conducted to evaluate nitrogen mineralization, nitrification and decomposition of organic carbon in soils amended with sewage sludge. Evolution of CO_2 from sludge-treated soils indicates that excessive amounts of trace metals may temporarily inhibit decomposition but that decomposition over an extended period of time tends to be independent of trace metals added. Some inhibition of nitrification was also observed in sludge-amended soils. There was a direct relationship between trace metals extracted with DTPA and total amount of metals applied to soils in sewage sludges. Sludge decomposition studies were conducted under laboratory and field conditions. The extent of sludge decomposition in soils was inversely related to the extent of treatment occurring within the sewage treatment plant. Carbon dioxide evolved from sludge-amended soils can originate from both the decomposition of organic C and the dissolution of inorganic carbonates present. A first-order model was evaluated to predict the extent of sludge decomposition under field conditions as a function of temperature and soil moisture. Laboratory incubation studies using dibutyl phthalate indicated that in excess of 90% of this compound will be degraded within 80 days after application to soils.

A series of studies were conducted to evaluate the uptake of cadmium, zinc, nickel, copper, iron, and manganese in oats, corn, wheat, and soybeans grown in soils treated with sewage sludge. Total metal concentrations were determined in whole grains and also in fractions determined by typical milling procedures. For oats, metal concentrations were significantly greater in the groats than in the hulls. Minimal metal uptake was observed when corn was grown on sludge-amended soils. The germ fraction of corn grain contained the highest metal concentrations. In both wheat and soybean grain, concentrations of Zn, Cu, Cd and Ni were increased by sludge applications with the bran and meal containing the highest concentrations for winter wheat and soybeans, respectively.

Soil solution samples obtained from soils amended with sludge were analyzed for metals and ligands and the data then used in a computer program to determine metal speciation. The speciation of zinc and cadmium into free ion and metal complexes was essentially the same in soil solutions regardless of total metal concentrations or the rate or frequency of sludge application. In general, copper and lead in soil solutions were significantly complexed by a model fulvic acid whereas cadmium, zinc, nickel and manganese were primarily present as inorganic complexes.

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SECTION 1

INTRODUCTION

The recent emphasis on applying sewage sludge to agricultural cropland necessitates a knowledge of the constituents contained in sludges and their fate in soils. Sludges can be employed as an alternative fertilizer material because they not only contain the macronutrients required by plants (i.e., N, P, and K) but also essential micronutrients (e.g., Cu, Zn, Mn). Numerous studies have shown that comparable crop yields can be obtained with conventional fertilizer materials and sewage sludge. However, sludges may also contain constituents that can be toxic to plants or to animals/man consuming products grown on sludge treated soils. Heavy metals and organics are the two broad groups of sludge constituents that have received the greatest emphasis in research and regulatory activities. Of the metals, cadmium appears to pose the greatest potential threat to human health while zinc, copper and nickel are of concern in decreasing crop yields. The group of orgainc compounds receiving the greatest attention is the polychlorinated biphenyls (PCB's).

This report describes the results obtained from a series of studies designed to (1) characterize the chemical composition of sewage sludges; (2) study the fate of sludge components in soils and; (3) evaluate the distribution of metals in crops grown on sludge treatd soils.

SECTION 2

CONCLUSIONS

1. The organic components in sludge were characterized by several extraction, chromatographic and spectroscopic techniques. Fatty acid esters (fats and waxes) and phthalic acid esters (plasticizers) comprised 2 to 10% and < 1% of sludge oven-dry weight, respectively. Palmitic acid esters and dibutyl phthalate were the predominant components in the respective fractions. Upon H_2SO_4 hydrolysis of the sludges and subsequent analysis of the hydrolyzates by high pressure liquid chromatography and colorimetric procedures, hexuronic acids, glucose, and xylose were detected. The sugars were likely monomers of hemicellulose and cellulose polymers which together comprised from 0.99 to 2.55% of sludge oven-dry weight. Organic N in the sludges was present primarily as α-amino acid N, comprising from 31 to 36.5% of the organic N. The amino acid distribution in sludge hydrolyzates as determined by an amino acid analyzer revealed the presence of amino acids of bacterial origin (i.e., α-amino pimelic, α,ε-diaminopimelic, β-alanine, γ-amino butyric and ornithine) as well as amino acids commonly found in plant and animal protein. Sodium-amalgam reduction of the sludges produced little or no phenolic compounds indicating small concentrations of humic type materials. Infrared and X-ray analysis of residues from HF-treated sludges and NaOH extracts (humic acids) of sludges indicated the presence of condensed aromatic rings or quinone-like compounds and little or no inorganic crystalline material.

2. To provide information on heavy metal associations in calcareous sludges, sludges were separated into light and heavy fractions by an ultrasonic dispersion and heavy liquid separation technique. The light fraction (organics) was 8 to 26% of the sludge dry weight while the heavy fraction (inorganics) comprised 69 to 88% of the sludge weight. The majority of the metals were associated with the sludge light fraction (organic). This separation technique should be useful as an initial step in the characterization of sludge properties and chemistry.

3. Infrared studies were conducted to qualitatively characterize sewage sludge light (organic) and heavy (inorganic) fractions obtained using a heavy liquid separation technique. Infrared spectra of sludge organic fractions revealed the presence of proteinaceous and polysaccharide-like materials. Infrared absorptions characteristic of carboxyl groups and aromatic compounds were not observed in the spectra of sludge light fractions. The position of the amide-I band in the infrared spectra of the sludge light fractions was used to obtain direct evidence of metal binding sites involving amide N and possibly amide O. A discrete shift

in the position of the amide-I band was initiated by extracting indigenous metal cations from the sludge organic fraction. This shift was reversed by saturating the extracted sludge organic fraction with Cu^{2+}. Absorption bands of quartz and calcite dominated the infrared spectra of sludge inorganic fractions.

4. Humic acids (HA) extracted from sludge, soil, and sludge-amended soil were characterized by elemental and functional group analyses and visible and infrared spectroscopy. When compared to Chalmers HA, the sludge HA appeared to be a more aliphatic, N-enriched polymer with decreased amounts of carboxyls and phenolic hydroxyls. Proteinaceous materials associated with the sludge HA appeared to be the major source of N enrichment. Sludge organic matter was apparently recovered in HA extracted from soil 2 and 14 months after sludge application. The association of sludge-borne proteinaceous and aliphatic materials with the sludge and sludge-amended soil HA fractions was indicated by increased amide and C-H stretch IR absorptions, as well as the increased N contents and decreased C:H ratios. The observation of proteinaceous materials associated with HA extracted from sludge-amended soil as long as 14 months after application suggested these proteinaceous materials were resistant to decomposition, possibly as a result of their incorporation into the HA structure.

   Electron spin resonance (ESR) spectroscopy was used to study the mechanism of Cu^{2+} binding by humic acids extracted from soil and sewage sludge. The ESR data presented showed that the addition of pyridine (py) and 1,10-phenanthroline (phen) to the soil and sludge Cu-HA complexes resulted in replacement of water coordinated to Cu^{2+} by the nitrogen ligands added. The added ligand did not displace HA oxygen donor ligands also coordinated to the Cu^{2+}. The addition of a more basic nitrogen donor, ethylenediamine (en), resulted in the displacement from Cu^{2+} of both coordinated aquo ligands and HA oxygen donors, forming $Cu(en)_2^{2+}$. ESR parameters revealed that two HA oxygen atoms were coordinated equatorially to Cu^{2+} in the original soil and sludge Cu-HA complexes. In the sludge Cu^{2+}-HA complex, the Cu^{2+} ion also appeared to form axial bonds with HA nitrogen donor ligands originating from proteinaceous materials associated with the sludge HA fraction. Characterization of an adduct with glycylglycine showed that the dipeptide formed coordinate bonds with Cu^{2+} bound to sludge HA.

   Infrared spectroscopy was used to identify the mode by which carboxylates of a soil HA compelxed Cu^{2+} and Fe^{3+}. Carboxylic acid groups of HA were converted to carboxylates (CO_2^-) by forming coordinate bonds with Cu^{2+} and Fe^{3+}. The separation of the antisymmetrical, $\nu_a(CO_2^-)$, and symmetrical, $\nu_s(CO_2^-)$, stretching vibrations of CO_2^-, obtained from spectra of metal-HA complexes indicated that CO_2^- formed a unidentate complex (i.e., a single M-O bond) with Cu^{2+} and Fe^{3+}. The formation of bidentate or bridging complexes between CO_2^- of HA and Cu^{2+} or Fe^{3+} was not observed.

5. Metal-chelate stability diagrams were developed as a function of pH and redox to predict the behavior of DTPA, EDTA, CDTA, EGTA, and HEDTA in soils. The metals evaluated include H^+, Fe^{3+}, Fe^{2+}, Al^{3+}, Mn^{2+}, Ca^{2+},

Mg^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, and Ni^{2+}. In acid systems, Ni^{2+} at 10^{-5} to 10^{-7} $\underline{M}$ was the predominant ligand species for all chelating agents. Similar results were obtained in alkaline systems when $NiCO_3$ controlled Ni^{1+} solubility. In the absence of Ni^{2+}, either Cd^{2+} or Pb^{2+} ligand species dominated at alkaline pH for all chelating agents. In acid systems, Cu, Pb, Fe and Zn are the major chelated species.

In acid soils, the chelating agents EDTA, EGTA, and HEDTA deserve further study as extractants for metals and as reagents for determining metal ion activities through competitive equilibria. The results identify ligands which may be useful in determining various metal ion activities in soils. The DTPA soil test appears to have a sound theoretical basis for evaluating plant available Pb, Cd, and Ni in soils.

6. A laboratory soil incubation system was used to study heavy metal extractability (DTPA), nitrogen mineralization, nitrification, and organic C decomposition in soils amended with municipal and synthetic sludges. The synthetic sludges were prepared to contain varying concentrations of Cu, Cd, Zn, Pb, and Ni at a constant level of organic C and N. Decomposition of municipal sewage sludges as measured by CO_2 evolution, appeared to be a function of the organic C content of the sludges. As much as 30 to 47% of the CO_2-C evolved from the sludge treated soils was due to dissolution of calcium carbonate present in the sludges. Significant inhibition of nitrification was found in soils amended with synthetic sludges at rates greater than 60 metric tons/ha, whereas stabilized municipal sludges showed rapid nitrification at all application rates. Generally, the percent of sludge organic N mineralized increased with sludge application rate. Heavy metals (Zn, Cu, Ni, and Cd) extracted from soil with DTPA generally increased with incubation time and were directly related to the amount of sludge-borne metals added to the soil. Similar correlations were obtained between added and DTPA extractable metals for synthetic and municipal sludges. In general, synthetic sludges offer promise for studying the rate of municipal sludges in soils.

7. The decomposition of liquid and dried municipal sewage sludges was determined in soils under laboratory and field conditions. Evolved CO_2 was continuously monitored in soil + sludge mixtures incubated in the laboratory. Primary, raw and waste activated sludges showed the greatest degree of decomposition while digested and composted sludges had the lowest decomposition values during a 112 day incubation. The data indicate that the percentage of sludge organic C decomposed in soil will be less for those sludges subjected to intensive stabilization processes at the treatment plant. Carbon dioxide evolution in the field study was monitored for a 24 hour period by sealing cylinders containing soil-sludge mixtures. The cylinders remained open to the environment between sampling periods. Soil moisture and temperature were also monitored. Evolution of CO_2 was initially rapid in all soil + sludge combinations but it decreased with time due to consumption of easily decomposable organic substrates in the sludge and gradual lowering of soil temperature in the late fall and winter. Carbon dioxide evolution rates for all treatments were affected by temperature and increased in

early spring. Dewatering an anaerobically digested sludge by air-drying reduced the extent of decomposition in soil. Inorganic C present in sludges can interfere with organic C decomposition calculations based on CO_2 evolution due to inorganic C dissolution. Data from both the laboratory incubation and field study show good agreement concerning relative order and magnitude of sludge decomposition. A first-order model proposed in current literature adequately predicts sludge decomposition under field conditions except for an undried sludge obtained directly from an anaerobic digestor. The model also failed to predict an increase in the rate of CO_2 evolution in early spring. This increase is possibly due to increased organic C availability induced by freezing and thawing cycles during the previous winter.

8. Incubation studies using carboxyl labelled ^{14}C dibutyl phthalate (DBP) added to soils at 0.1% (w/w) indicate that 90% of the DBP was degraded within 80 days. Decomposition of DBP was shown to be microbially mediated and was a function of soil type, pH, and temperature. Application of DBP at rates of 0.4% (w/w), and the addition of ammonium and $CaCO_3$ or sewage sludge had little effect on the extent of DBP degradation. The addition of DBP in sewage sludge or other waste materials to soils should not pose a long-term persistance problem.

9. A study was initiated to determine the retention capacities of calcareous and acidic sludges, sludge fractions, and extracted sludges for Cd, Cu, Ni and Zn. The results obtained from the equilibration of Cd, Cu, Ni or Zn solutions with calcareous and acidic sludges indicated that calcareous sludges have a higher capacity to retain added metals than noncalcareous sludges and that metal retention was directly related to sludge pH. As solution pH increased above 5.0, chemical precipitation of metals, as carbonates, becomes the dominant mechanism in the removal of Cd, Cu, Ni or Zn from solution. Metal retention was reduced when pH values of sludge-metal solutions were below 5.0. A majority of the Cd, Cu, Ni and Zn retained by sludge at low pH values was likely due to complexation by organic matter and sorption by amorphous Fe, Al and Mn hydrous oxides. The following trend in retention capacity was observed after equilibration of Cd and Cu solutions with the heavy and light fractions of two calcareous sludges: light fraction > heavy fraction > whole sludge. This finding also suggests that organic materials in sludge form stable complexes with metal ions. The heavy fraction which is composed largely of inorganic materials also retained large proportions of added Cd and Cu. This finding suggests that carbonates in sludge precipitate added metals and that complexation of Cd and Cu by hydrous oxides of Fe, Al and Mn may play an important role in metal retention by sludges. The sludge components primarily responsible for Cd, Cu, Ni or Zn retention were determined by selective extraction procedures. The results indicated that for a given metal there are three sludge components involved in Cd, Cu, Ni or Zn retention. Sludge components ranked in decreasing order of importance for retention of each metal are: Cd, carbonate > organic matter > hydrous oxides; Cu, carbonate > organic matter = hydrous oxides; Ni, carbonate > organic matter = hydrous oxides; Zn, carbonate > organic matter > hydrous oxides.

10. Oats, corn, wheat and soybeans were grown on soils treated with stabilized sewage sludge from three Indiana cities. Sludges were applied to a Chalmers silty clay loam soil at rates ranging from 56 to 448 metric tons/ha. Oat samples were separated into groats and hulls and concentrations of Cd, Zn, Cu, Ni, Fe, and Mn were determined in each fraction by atomic absorption spectrophotometry. The effect of sludge application rate on the concentration of trace metals in whole oats, groats and hulls was similar for all three sludges used and, in general, decreased in the following order: Zn = Fe > Mn > Ni > Cu > Cd. Except for Mn, the concentrations of trace metals increased with increasing rates of sludges applied. However, the groats contained greater concentrations of Cu, Ni, Zn, Cd and Mn than the hulls.

    The metal concentrations in whole kernel corn were not significantly increased by applying from 56 to 448 metric tons of sewage sludge per hectare. Except for one Cd-enriched sludge, Cd concentrations in whole corn and fractions from dry-milling were < 0.05 mg/kg. As expected, the germ fraction contained the greatest proportion of the fat, ash, and trace metals while the majority of the protein was found in the grits fraction. Both the concentration and percent distribution of metals in dry-milling fractions decreased in the following order: germ > hulls > flour > meal > grits. The metal concentrations in both wheat and soybean grain were increased by sludge applications. For wheat, the bran contained significantly higher concentrations of all metals than the flour. Metal concentrations were similar in whole soybean grain and the resulting meal.

11. An experiment was initiated by the Tennessee Valley Authority in 1971 to determine the effects of land applications of sewage sludge on the heavy metal status of a soil. Sewage sludge from Tuscumbia, Alabama was applied to an acid soil (pH $\sim$ 5) as a single treatment in 1971 or as annual treatments from 1971 through 1974 in amounts totaling from 50 to 800 metric tons ha^{-1}. Soil samples (0- to 15-cm depth) obtained from the plots in 1977 were moistened to -0.33 bar water potential and equilibrated for 7 days prior to displacement of the soil solution. The soil solutions were analyzed for Zn, Cd, Cu, Ni, Pb, Mn, Fe, Ca, Mg, Al, Na, K, PO_4, SO_4, Cl, conductivity, pH, and organic C, and the metal species present were calculated with the computer program GEOCHEM. Application of sludge increased soil solution concentrations of Zn, Mn and Cd while Cu, Ni and Pb were present in most solutions at levels below the detection limits of the analytical methods used. Cadmium, Zn, and Mn in the soil solution appeared to exist predominately as the free ion. Complexation by inorganic and organic ligands ranged from 9 to 33%, 3 to 22% and 3 to 31% of the total soluble Cd, Zn and Mn, respectively, depending on the model used to represent metal interaction with soluble organic C. Cadmium was complexed with organic ligands to a greater extent than were Zn or Mn. Sulfate, phosphate, and chloride formed inorganic complexes with Cd, Zn, and Mn, but they constituted < 10% of the total metal present. The general degree of metal complexation by organic and inorganic ligands increased in the order: $Zn \cong Mn \cong Ni < Cd < Cu$. Speciation of Zn and Cd into free metal ion and complexed forms was essentially the same in the soil solution regardless of metal concentrations in solution and rate or frequency of sludge

application. calculated activities of Cd^{2+} indicated undersaturation with respect to known Cd solid phases while Zn^{2+} activities were comparable to those for $ZnFe_2O_4$, soil-Zn, and Zn_2SiO_4. Soluble Zn and Cd were directly related to the amount of metal added and to the extractability of added metal for DTPA. The solubility and DTPA extractability of Cd were increased to a greater extent by a single application of sludge than by multiple, annual additions; this relationship was not as consistent for added Zn.

SECTION 3

CHARACTERIZATION OF ORGANIC COMPONENTS IN SEWAGE SLUDGE

INTRODUCTION

The elemental composition of municipal sewage sludges has been studied by several investigators (Berrow and Webber, 1972; Berggern and Oden, 1972; Blakeslee, 1973; Sommers et al., 1973; Sommers et al., 1976; and Sommers, 1977) and the inorganic crystalline components in sludges have been examined by Silviera et al. (1977). These investigations have established that organic materials comprise from 40 to 60% of sewage sludge dry weight. Recent studies have demonstrated that 40 to 84% of the total N, from 12 to 67% of the total P, and from 29 to 76% of the total S in sludges are present in organic compounds (Sommers et al. 1973; Sommers et al., 1977). A high proportion (~90%) of organic N in sludges is solublized during 6N HCl hydrolysis and ~ 50% of the hydrolyzable N is α-amino acid N suggesting that a significant amount of sludge N is in the form of peptides or proteins. A low (< 3%) proportion of sludge organic N is present as hexosamines (Sommers et al., 1973). The organic S in sludges is largely present as carbon-bonded S (e.g., methionine and cysteine) and ester sulfate S such as choline sulfate, phenolic sulfates, or sulfated polysaccharides (Sommers et al., 1977).

Studies of the organic materials in raw sanitary wastewater have indicated the presence of grease, fat, protein, carbohydrates, lignin, amino sugars, and organic acids (Hunter and Heukelekian, 1965; Painter, 1971). Investigations have suggested that anaerobically-digested sewage sludges contain humic acids, hemicellulose, cellulose, proteins, lignin, and water-soluble organic materials (Heukelekian, 1930; Heukelekian, 1958; McCoy, 1971). Wiswanathan et al. (1962) have reported that the lipid components present in digested sludge were fatty acid esters and that the predominant species of fatty acids were palmitic, stearic, oleic, and linoleic acids. Other studies have shown that palmitic and stearic acids and their esters accounted for more than 80% of the saturated fatty acid content of sludges (Mueller, 1955). Sludges are routinely extracted with a nonaqueous solvent (e.g., hexane) to estimate the amount of oil and grease present (American Public Health Assoc., 1971).

The presence of large concentrations of fats and oil may have significant effects upon decomposition processes and nitrogen transforations following the addition of sludge to soil. For example, if inorganic N were immobilized during degradation of fats and oils in soil, the annual sludge loading rate may be increased to compensate for short-term decrease in plant available N. Beauchamp et al. (979) and Terry (1976) have reported little mineralization and/or initial net immobilization during decomposition in soil of sludge containing relatively high levels (7.5%) of hexane-soluble compounds. Hohla *et al.* (1978) reported that grease and oil resist decomposition following addition of sludge to soil resulting in an alteration of soil physical properties.

The objectives of this research were to determine the amounts of materials present in representative sludges that were extractable with nonaqueous solvents and to characterize the types of organic compounds in this sludge fraction. The approach used was to extract the sludge with hexane and other nonpolar solvents followed by characterization of the compounds in the extract by chromatography, infrared and nuclear magnetic resonance spectroscopy, and mass spectrometry.

MATERIALS AND METHODS

Sludges

Bulk samples of anaerobically-digested sewage sludge were obtained from sewage treatment plants in eleven Indiana cities. Descriptions of the treatment plants sampled have been previously given (Sommers *et al.*, 1976). In general, solids subjected to anaerobic digestion were derived from primary and secondary stages of wastewater treatment. Sludge samples from Anderson, Crawfordsville, Gary, Kokomo, Lafayette, Lebanon, Peru, Tipton, and Warsaw, Indiana were taken directly from the anaerobic digesters, placed in sealed glass containers, and stored at 4°C. Samples of partially dried anaerobically-digested sewage sludge were also obtained from sand drying beds at sewage treatment plants at Anderson, Marion, and Frankfort, Indiana. The partially dried sludges were treated in the same manner as the liquid samples. A subsample (~ 200 g dry weight) of each sludge was air-dried at 21°C, ground to pass a 60-mesh seive, stored in a sealed glass container at 21°C, and used for all analyses.

Analytical Methods

Methods used to characterize the sludges (Table 3.1) were those described by Sommers *et al.*, (1973) and Sommers *et al.*, (1977). The percent ash was determined gravimetrically by a dry ashing procedure (650°C for 2 hours). The organic C, total N, NH_4^+-N, total P, and total S concentrations of each sludge were determined by the methods of Allison (1960), Nelson and Sommers (1972), Bremner (1965), Sommers and Nelson (1972), and Tabatabai and Bremner (1970), respectively.

Nonaqueous solvent extraction--

Total amounts of nonaqueous solvent soluble constituents in sludges were determined by sequential extraction (24 hours) of 15 g of sludge with 150 ml each of hexane and methanol using a Soxhlet apparatus, evaporation of the solvent, and gravimetric measurement of extracted organics. The organic C content of extracted oil and grease was estimated by the Allison (1960) procedure. Samples (15 g) of Anderson, Frankfort, and Marion sludges were sequentially extracted for 24 hours with 150 ml each of hexane, benzene, acetone, and methanol using a Soxhlet extractor. The samples were rapidly dried (50°C) after each extraction and the amount of material removed was determined by differences in weight before and after extraction. Samples (15 g) of Anderson, Frankfort, and Marion sludges were also extracted for 24 hours with 150 ml of a hexane, methyl ethyl ketone, methanol (1:2:1, v/v/v) mixture using a Soxhlet apparatus. The amount of organic material extracted was determined by differences in weight of dried (50°C) sludge samples before and after extraction. Varying extraction procedures were used in an attempt to verify that the solvent systems used quantitatively extracted the oil and grease fraction from sludges. All organic solvents used were analytical reagent grade.

Organic compounds in hexane, benzene, acetone, and methanol extracts of Anderson, Frankfort, and Marion sludges were separated by thin layer chromatography and qualitatively examined by a Perkin-Elmer model 421 infra-red spectrometer and a Varian A-60A nuclear magnetic resonance spectrometer. The compounds in the mixed solvent extract were also qualitatively and semiquantitatively analyzed by gas chromatography-mass spectrometry (GC-MS) using a Finnigan 4000 GC-MS equipped with a model 6100 data system. A temperature programmable GC was used with either a 2 meter column of 3% OV-3 on Gas-Chrom Q or a 2 meter column of Silar 10C on Gas-Chrom Q depending upon the sludge being analyzed. Organic solvent blanks were carried through the extraction procedures and analyzed by GC-MS. The relative amounts of the major organic compounds extracted were determined by quantitating the peak areas of the gas chromatogram recorder tracings. Solutions containing known concentrations of dibutyl phthalate, di(2-ethythexyl) phthalate, butyl benzyl phthalate, and n-propyl palmitate were used as standards.

Carbohydrates--

Air-dry samples (15 g) of anaerobically-digested sewage sludges from Anderson, Marion and Frankfort, Indiana, were sequentially extracted with hexane (150 ml) and benzene (150 ml) for a total of 48 hours in a Soxhlet extractor. The solvent-extracted samples were then subjected to three different hydrolysis procedures to assist in selection of a method to maximize liberation of monomeric carbohydrates from polymers, and to minimize destruction of released monosaccharides.

The first procedure used was an adaptation of the one used by Oades *et al.* (1970) for the determination of carbohydrates in soils. Duplicate samples (2.5 g) of each of the three sludges were refluxed for 20 minutes with 2.5 *M* H_2SO_4. The samples were filtered while warm through a sintered glass funnel and the residues were washed with deionized water. The filtrate and combined washings were neutralized to pH 7 with excess $SrCO_3$ and allowed to stand at room temperature until all of the brown organic-iron complexes had precipitated out of solution. The sludge residues were then soaked for 26 hours in 13 *M* H_2SO_4, filtered, and the filtrate neutralized with $SrCO_3$. The sludge residues remaining after the 26 hour strong acid soak were refluxed for 3 hours with 0.5 *M* H_2SO_4 and the resulting solution was filtered while warm through a sintered glass funnel. The filtrate was neutralized with excess $SrCO_3$. The three neutralized filtrates were filtered to remove the insoluble Sr salts and combined. The combined filtrates were evaporated to dryness on a rotary evaporator at reduced pressure and extracted with four 10 ml portions of methanol to separate the monomeric sugars from the inorganic salts. After removing the methanol with a rotary evaporator, the resulting precipitate was dissolved in deionized water and diluted to volume (10 ml). The carbohydrate solutions were kept refrigerated until analyses could be performed.

The second procedure evaluated involved refluxing 2.5 g of sludge with 25 ml of 0.5 M H_2SO_4 for 2 hours, filtering the samples while warm through a sintered glass funnel, and preparing the filtrate for carbohydrate analysis by using the neutralization, evaporation, and methanol extraction procedures described above.

The third procedure evaluated was an adaptation of Browning's method (Browning, 1967) for carbohydrate analysis in woody materials. Air-dry samples (0.1 g) of the extracted sludges were weighed into Pyrex test tubes (50 ml). After adding 0.3 ml of 11.5 M H_2SO_4 to each sample, the test tubes were placed in a 35°C water bath for 30 minutes. Seven ml of deionized water were added to each sample mixture and the test tubes were refluxed in a 100°C glycerol bath for 0.5, 1.0, 2.0, 3.0, 4.0, and 4.5 hours. A sample of each sludge was removed at each of the designated times and filtered through a sintered glass funnel under suction into a 10 ml volumetric test tube. The filtrate was diluted to volume and neutralized with excess $BaCO_3$. The resulting suspension was allowed to settle undisturbed for 2 days causing three layers to form. A portion of the middle layer was removed for analysis.

Hydrolyzates prepared by all three procedures were analyzed for monosaccharides by a Waters High Pressure Liquid Chromatograph (HPLC) equipped with a column capable of separating simple carbohydrates (Ladisch et al., 1978). A 0.6 x 30 cm column containing Aminex 50 W x 4 cation exchange resin was used for separation and a refractive index detector was used to quantitate the monosaccharides. The eluting solvent was deionized water at 85°C with a flow rate of 0.6 ml/min.

An anthrone reaction (Dische, 1962) was used to estimate the total amounts of carbohydrates present in the sludge hydrolyzates. An aliquot of the hydrolyzate containing less than 100 µg of total carbohydrate was placed in a 20 ml Pyrex test tube. Enough distilled water was added to the aliquot to give a total volume of 2 ml. The anthrone reagent was prepared by adding 5 ml of absolute ethanol and 100 mg of anthrone to a 100 ml volumetric flask and diluted to volume with 12.75 M H_2SO_4. The reagent mixture was shaken until dissolved and was freshly prepared before use. The test tubes and the anthrone reagent were cooled in an ice water bath for several minutes. Each test tube was shaken vigorously in the ice water bath as 10 ml of anthrone reagent was added. After leaving the treated test tubes in the ice water bath for several minutes, the test tubes were transferred to a vigorously boiling water bath. Ten minutes later the test tubes were returned to the ice water bath. After the test tubes had cooled, the absorbance of the colored solution was determined on a Beckman Model 24 spectrophotometer at a wavelength of 625 nm. The sample concentration was determined by comparison to a standard curve obtained from addition of 0, 25, 50, 75, and 100 µg glucose to separate test tubes before addition of anthrone reagent. The standard glucose solutions were prepared by making a 500 mg/l stock solution in 0.15% benzoic acid and diluting a portion of the stock solution as needed.

The hexuronic acid content of the sludge hydrolyzates was determined by a carbazole-H_2SO_4 reaction (Dische, 1947). An aliquot of sludge hydrolyzate containing less than 50 μg of hexuronic acids was placed in a 20 ml Pyrex test tube. All samples were diluted with deionized water to give a total sample volume of 0.5 ml. Three milliliters of concentrated H_2SO_4 were added to the samples and the treated samples were refluxed in a 100°C bath for 20 minutes. After the treated samples had been cooled with tap water for 5 minutes, 0.1 ml of a 0.1% carbazole in ethanol solution was added as the tubes were shaken. The samples were allowed to stand for 2 hours at 25°C to allow for full color development and the absorbance of the solutions were determined at a wavelength of 535 nm on a Beckman Model 24 spectrophotometer. The amount of hexuronic acid present in the aliquot was estimated by comparison of the sample's absorbance to absorbance values obtained when 0, 12.5, 25, 37.5, and 50 μg of glucuronic acid were added to test tubes for use as a standard curve.

The glucose content of the sludge hydrolyzates was determined by a specific glucose oxidase exzyme analysis using a Beckman Glucose Analyzer. The amount of glucose present was determined by the amount of oxygen consumed as compared to that for glucose standards.

The orcinol reaction with pentoses (Albaum, 1947) was used for the determination of the xylose concentrations in sludge hydrolyzates. A 100 μl aliquot from each of the neutralized sludge hydrolyzate samples was placed in a 20 ml Pyrex test tube and diluted to a total volume of 1 ml with deionized water. The orcinol reagent was prepared by mixing 8 ml of a 10% solution of orcinol in ethanol and 80 ml of a 0.1% solution of ferric chloride hexahydrate in concentrated HCl. The orcinol-ethanol solution was stored at -20°C to prevent discoloration. The orcinol reagent mixture was freshly prepared before use to keep darkening of the reagent to a minimum. After 1 ml of orcinol reagent was added to the diluted samples, the test tubes were placed in a 109°C glycerol bath and refluxed for 40 minutes. A glass marble was placed on the top of each test tube to reduce water evaporation. After the samples had refluxed for 40 minutes, they were placed in a 25°C water bath for 10 minutes. Three milliliters of deionized water were then added to each of the samples. After the contents had been thoroughly mixed, the absorption intensities of each of the resulting solutions were recorded at 666 and 583 nm using a Beckman DU spectrophotometer. The pentose concentrations in the sludge hydrolyzates were determined by reference to a standard curve which was developed by using known concentrations of xylose and the colorimetric procedure described above. By subtracting the absorbance of the pentose-orcinol reaction product at 583 nm from the absorbance at 666 nm any interference from hexoses present in hydrolyzates can be eliminated. The net absorbance is due only to furfural-forming substances which consist of the pentoses and the hexuronic acids (Meulberg, 1976). Since the extinction coefficient for the hexuronic acids is one-half the value for the extinction coefficient of the pentoses the following equation may be used:

$$[\text{furfural formers}] = [\text{pentoses}] + 1/2\ [\text{hexuronic acids}] \qquad (1)$$

The orcinol reaction estimated the total concentration of the pentoses plus the hexuronic acids present, whereas the carbazole reaction estimated the hexuronic acid concentration. The pentose concentration in sludge hydrolyzates was therefore calculated by use of Equation 1 and known values for hexuronic acid concentration and total furfural-forming compounds.

Organic Nitrogen--

The organic N content of sludges was estimated as the difference between total N (Nelson and Somemrs, 1972) and inorganic N contents (Bremner, 1965a). The distribution of organic nitrogen compounds in sludge was determined according to the method of Bremner (1965b). Sludge samples (400 mg) were hydrolyzed under reflux for 12 hours with 15 ml of 6 M HCl. Hydrolyzates were filtered and residues were washed with 6 M HCl. After neutralization of the combined filtrate and washings, the ammonium-, amino sugar-, and amino acid-N contents in the hydrolyzates were determined by steam distillation. The nonhydrolyzable N in the hydrolysis residue was divided into NaOH-extractable N (that N present in humic materials) and into residual N. The humic acid N and residual N values were determined by performing a total N analysis on the appropriate fractions. The amount of hydrolyzable protein was calculated by dividing the amount of α-amino acid N detected in sludge acid hydrolyzates by 0.13.

For quantitative estimation of amino acids present, a 300 mg sample of anaerobically-digested sewage sludge which had been previously extracted with organic solvents was refluxed in 20 ml of 6 M HCl for 16 hours. After the amino acids had been purified (Stevenson, 1965), the resulting aqueous solution containing the amino acids was evaporated to dryness at 50 to 60°C under reduced pressure. The amino acid residue was dissolved in a pH 2.6 citric acid buffer. A 0.5 ml aliquot of the buffer solution containing a known amount of added norleucine used as an internal standard was analyzed on a Technicon Amino Acid Auto Analyzer. The amount of each amino acid present was determined by comparing the peak areas of the amino acids in the samples to those of respective standards. Norleucine was also used as an internal standard in the amino acid standards solution.

Nonhydrolyzable Organic Fraction--

Samples of anaerobically-digested sewage sludges were used in the analysis procedures to determine the organic compounds present in the humified material. The sludge samples were previously extracted with organic solvents and refluxed for 12 hours in 6 M HCl to eliminate any possible interferences from carbohydrates, amino acids, acid soluble metals, or organic solvent soluble compounds during analysis of the humified organic matter. Analysis of the humic material proved to be very difficult. Three different procedures were used in determining the types of organic compounds present in the humified fraction of anaerobically-digested sewage sludge.

The first procedure performed involved a Na-amalgam reduction of a 100 mg sludge sample followed by two-dimensional thin layer chromatography of the ether-soluble products. The Na-amalgam reduction procedure was performed according to the method of Martin et al. (1974) except for a few minor variations. A 4% Na in Hg mixture was prepared instead of the 5% mixture used in Martin's work. The 4% Na in Hg mixture was prepared by dissolving 12 g of Na a small portion at a time in 300 g of Hg. The Hg had been previously weighed into a 250 ml beaker and covered with a 2 cm layer of mineral oil to insure that the Na-amalgam would not be exposed to the air during its formation. It was necessary to use a pair of pliers to crush the Na-Hg solid mixture that formed after each Na addition to facilitate a proper reaction between the entire 12 g of Na and the Hg. After all of the Na had been added, the mineral oil was removed. The amalgam was washed several times with anhydrous benzene, ground to a coarse powder in a stainless steel grinding mill, and washed with peroxide-free anhydrous ether. The excess ether was poured off and the remaining ether was removed by gently warming the amalgam which had been placed in a large evaporating dish in the hood. The dried amalgam was then immediately placed in a bottle and stored in a desiccator until it was used.

The Na-amalgam reduction apparatus was assembled as in Figure 3.1. The pyrogallol O_2 traps were prepared by adding 15 g of pyrogallol and 75 ml of H_2O to each flask. The two flasks, which are in series, were flushed with N_2. Then, 25 ml of H_2O containing 8 g of NaOH were added to each flask through the fitted separatory funnels. After preparation, the O_2 trapping flasks were sealed tightly when not in use. Pyrogallol was later added to the O_2 trapping flasks at various time intervals to regenerate the O_2 trapping capabilities of the system. The Na-amalgam (25 g) was added to the reaction vessel by removing the separatory funnel. This step was performed as quickly as possible to keep the O_2 contamination of the system to a minimum. After the reaction had been completed and the digestion mixture cooled in an ice bath, sufficient 6 M HCl was added via the separatory funnel to adjust the pH of the flask contents to 1.0. The two-dimensional thin layer chromatography system used was the same as that of Martin et al. (1974). Diazotized sulfanilic acid was used as the spray reagent (Stahl, 1965). The phenols appeared as yellow to reddish colored spots on a white background. The entire system was tested on several model phenolic polymers and was found to work very well.

The second procedure used in determining the basic constituents of the humified organic fraction involved a 1 M NaOH extraction of the pretreated sludge followed by a Na-amalgam reduction of the NaOH-soluble extract. Forty milliliters of 1 M NaOH was shaken with 2 g of sludge for 16 hours. The mixture was filtered and the residue was washed with a small volume of deionized water. The filtrate was adjusted to pH 1 with HCl and allowed to sit undisturbed for 24 hours. The humic acid precipitate was collected by suction filtration on a previously tared sintered glass funnel, washed with a small volume of deionized water, and freeze-dried. The funnels were weighed, and the amount of humic acid extracted was determined by the difference in weight. Thirty-five milligrams of the sludge humic acid were treated with the Na-amalgam and analyzed by two-dimensional thin layer chromatography as described above.

The third method of analysis of the residue involved a 1 M HCl-5 M HF digestion (Leenheer and Moe, 1969) of a pretreated sludge sample. Thirty milliliters of the HCl-HF solution was shaken with 1.5 g of sludge for 24 hours in a centrifuge tube. The mixture was then centrifuged and the HCl-HF solution was decanted. The sludge was washed with 1 M HCl, shaken vigorously, and centrifuged. The 1 M HCl was decanted, and the entire extraction procedure was repeated twice to result in a total of 3 HCl-HF extractions on each sludge sample. After the third HCl-HF extraction, the sludge residue was suction-filtered on a sintered glass funnel, washed with a small volume of deionized water, and freeze-dried. Duplicate samples of each sludge before and after the HCl-HF extraction were dry-ashed in a muffle furnace at 650°C for 2 hours to determine the amount of mineral matter that was destroyed. X-ray analysis was also performed on the HCl-HF extracted sludge samples to determine if any crystalline mineral matter remained after the HF treatment. A Siemens Type F X-Ray Machine with an Ortec Model 6713 axis controller containing a digital drive was used to obtain the X-ray spectra. The NaOH soluble sludge organic matter remaining in HCl-HF treated residues was extracted by the method described above and 35 mg of the resulting humic acid was treated with Na-amalgam and analyzed by two-dimensional thin layer chromatography according to the method previously described. Infra-red studies were also performed on the humic acids and the NaOH insoluble sludge residues. The samples were prepared as a KBr disk, and the spectra were recorded on a Perkin-Elmer Model 180 infra-red spectrometer.

Previously weighed air-dry samples of anaerobically-digested sewage sludges were sequentially extracted with hexane, benzene, acetone, and methanol for 24 hours in a Soxhlet extractor. After the residues had been air-dried and weighed, portions of the sludges were refluxed in 6 M HCl for 12 hours. The nonhydrolyzable material was quantitatively transferred to a previously tared sintered glass filter funnel, sunction filtered to remove any hydrolyzable proteins and carbohydrates, oven-dried, and weighed. The organic carbon distribution in the original and treated sludge samples was determined by subtracting the amount of CO_2-C liberated during an H_2SO_4-$FeSO_4$ digestion (inorganic C only) from the amount of CO_2-C liberated during a H_2SO_4-H_3PO_4-K_2CrO_7 digestion (total C) (Allison, 1960). The liberated CO_2 was trapped in 10.00 ml of 1 M KOH. Excess 1.5 M $BaCl_2$ was then added to the KOH solution to precipitate the CO_2 as $BaCO_3$, and the unreacted KOH was back-titrated with standardized 0.473 M HCl to a phenolphthalein endpoint. The percent carbon in a given sample was determined by equation 2.

$$\%C = \frac{(\text{ml tit. blank} - \text{ml tit. sample}) \times 6 \times \underline{M}\ \text{HCl} \times 100\%}{\text{mg of sample}} \qquad (2)$$

Duplicate samples and blanks were used throughout the entire procedure. All carbon values obtained were converted to be expressed as a percent of the initial oven-dry weight of the sludge.

All data reported are on a moisture-free basis; moisture being determined from loss in weight following drying at 105°C for 24 hours. All analysis were conducted in duplicate and data reported are average values.

RESULTS AND DISCUSSION

Sludges collected from anaerobic digesters (Table 3.1) contained ash (avg 53%), organic C (avg 23.8%), organic N (avg 2.02%), total P (avg 2.10%), and total S (avg 1.50%) concentrations similar to those previously reported for anaerobic sludges (Sommers, 1977; Sommers *et al*., 1977).

However, sludge samples obtained from sand drying beds contained higher ash (avg 67.5%) and lower organic C (avg 12.4%) concentrations than liquid anaerobic sludges (Table 3.1). The organic N and total S concentrations of drying bed sludges were also lower than those for liquid sludges. When organic C concentrations were expressed on an ash-free basis the average values for liquid anaerobic and drying bed sludges were 51.1 and 39.2%, respectively. Two explanations are possible for the higher ash and lower organic C concentrations in drying bed sludges. First, organic matter in sludge may be diluted by sand used as the filter material in the drying beds. Secondly, the extended period of aerobic decomposition allowed by the drying bed treatment may result in reduced organic matter levels in sludges.

The NH_4^+-N levels in both types of sludges were low as compared to previous reports (Sommers et al., 1973; Sommers, 1977) because of extensive NH_3 volatilizaton during air-drying of sludges. The total P concentrations and organic C:organic N ratios were similar in liquid anaerobic and drying bed sludges. These findings were expected because sludge decomposition would not alter the total P concentration and probably not change the organic C:organic N ratio because both constituents could be mineralized at similar rates.

Extractability of Oil and Grease

Significant amounts (8.7% of dry weight; 20.2% of ash-free dry weight) of organic components were extractable from all sludges with hexane (Table 3.2). The proportion of total volatile solids in sludges extracted by hexane varied from 5.1 to 47.7%. This finding indicates that the quantities of oil and grease in sludges are highly variable and likely reflect both input of these components into the sewage treatment plants and their relatively slow decomposition during anaerobic digestion. Similar variability has been observed for other sludge constituents (Doty et al., 1978; Sommers et al., 1976; Sommers, 1977). Small amounts (1.7% of dry weight; 4.0% of ash-free dry weight) of organic materials are removed from hexane-treated sludges by methanol extraction (Table 3.2). Methanol extracted from 1.1 to 5.9% of the volatile solids indicating that the amounts of methanol-soluble compounds in sludges are not as variable as the amounts of hexane-soluble constituents. Extraction of hexane and methanol treated drying bed sludges with benzene and acetone increased the total recovery of nonpolar solvent soluble materials by 10 to 56%; however, the absolute amounts of constituents removed by these solvents were only 3 to 9 mg/g of sludge.

Total recovery of organic materials from sludges by hexane + methanol or hexane + methanol + benzene + acetone varied from 36 to 252 mg/g of sludge (Table 3.2). The proportion of sludge volatile solids recovered by the extractants varied from 9.6 to 52.5% (avg 24.9%). Extraction of drying bed sludges with a mixed solvent system (hexane:methyl ethyl ketone:acetone) gave recoveries of organic materials similar to the total of those obtained with sequential extractants. These findings suggest that sludges contain significant amounts of hydrophobic compounds soluble in nonaqueous solvents and that sequential treatment with hexane and methanol will extract most of the oil, grease, and related compounds present in sludges.

Nonaqueous solvents (hexane + methanol or hexane + methanol + benzene + acetone) extracted from 24 to 170 mg organic C per g sludge (Table 3.3). The proportion of sludge organic C recovered in nonaqueous solvent extracts varied from 21 to 71% (avg 36%). A larger percentage of sludge organic C was extracted by nonpolar solvents as compared to the proportion of volatile solids recovered. Therefore, the constituents extracted must have a higher carbon content than the overall sludge organic components. Only trace amounts of total N were found in hexane, methanol, and mixed solvent extracts. These data suggest that nonaqueous solvents were extracting only oil, grease, and fats and that carbohydrates or proteins were not co-extracted.

Organic Compounds in Extracts

To determine the nature of hydrophobic compounds in sludges, non-aqueous solvent extracts from drying-bed sludges were analyzed by chromatographic and spectrophotometric techniques. Dry bed sludges were selected for detailed analysis because they were being used in large scale field studies of plant response to sludge addition and have been used in several laboratory incubation experiments. Furthermore, preliminary GC analysis of hexane extracts suggested that sludges from sand drying beds and anaerobic digesters contained similar compounds.

Of the material removed by the mixed solvent Soxhlet extraction procedure, GC analysis indicated trace or greater amounts of 30, 37, and 20 organic compounds in extracts from Anderson, Frankfort, and Marion sludges, respectively. These compounds were characterized as much as possible by nuclear magnetic resonance, infrared, and mass spectrometry. Because of incomplete resolution by the chromatographic methods employed, more than one compound may have been present in some of the fractions. Quantitation or specific identification of each compound in the sludge extracts could not be completed because of the complexities of the resulting spectra. However, the spectra obtained were characteristically aliphatic, suggesting that most of the unidentified compounds are hydrocarbons or fatty acids and their esters.

A sample GC recorder tracing and the corresponding MS fragmentation patterns of the major compounds in the extract from the Frankfort sludge are given in Figure 3.2. Similar results were obtained from the other sludge extracts examined. It was difficult to identify all compounds in the sludge extract because of incomplete resolution by the GC column. However, five major components (three phthalate esters, one phenolic fatty acid ester, and one wax) were readily separated and identified in the Frankfort extract. The phthalates are discernable because of their strong fragmentation peak at a m/e value of 149 and the phenolic fatty acid ester and wax were characterized on the basis that their fragmentation patterns were characteristically aliphatic. Thin layer chromatography followed by infrared spectroscopy was used to confirm the identities assigned to compounds present in extracts.

The major nonaqueous solvent soluble compounds found to be present in anaerobically-digested sludge are given in Table 3.4. From 3.2 to 11.7% of the total sludge oven-dry weight was composed of fatty acid esters with the palmitic acid esters being the major constituents of this fraction. Several other types of fatty acids and their esters (fats and waxes) were also found to be present in each sludge extract. The fats and waxes were found to consist of variable molecular weight alcohols bonded in ester linkages to palmitic, stearic, oleic, linoleic, and other fatty acids. It is believed that the origin of these compounds are from natural sources. This data, along with the work done by Balmat (1955), Heukelekian (1958), and Wiswanathan et al. (1972) strongly indicate that fatty acid-related compounds compose 2 to 12% of the total oven-dry weight of the anaerobically-digested sewage sludges studied.

The GC-MS data have also shown that significant concentrations (120 to 600 μg/g of dry weight) of phthalate esters were present in all three sludges. The sludges used were not exposed to any known phthalate source after collection indicating that phthalates are a sludge constituent. Some doubt has been raised as to whether phthalate esters are natural products or pollutants (Mathur, 1974). Phthalate esters are used extensively in plastics, cosmetic, fragrances, pesticide carriers, industrial oils, munitions, and insect repellents. The United States in 1973 produced 9.98 x 10^9 kg of phthalate esters and this amount is expected to increase tenfold in less than 30 years (Anonymous, 1973).

Phthalate esters have also been found to naturally occur in cigar smoke, tobacco leaves, grapes, cranberries, poppies, oxidized corn oil, soil, and crude oil (Anonymous, 1972). Several investigators (Kahn and Schnitzer, 1971, 1972; Neyroud and Schnitzer, 1974; Schnitzer, 1974; Schnitzer and Neyroud, 1975; Schnitzer and Ortiz De Serra, 1973; Schnitzer and Skinner, 1974) have reported the presence of dialkyl phthalates in model fungal humic and fulvic acids. approximately 16% of one fungal (Stachybotrys chartarum) humic acid was composed of phthalate esters indicating that microorganisms are capable of making phthalate esters from phthalic acid and long chained alcohols (Schnitzer and Neyroud, 1975). Several analytical techniques have been used to confirm the presence of phthalate esters in fungal humic acids. The organic solvents used in these fungal studies were carefully purified, thus eliminating the possibility of phthalate contamination from an external source.

Even though significant amounts of fatty acid related compounds and phthalate esters have been extracted from the sludges, there is evidence to suggest that a portion of these materials may still remain in the sludge after exhaustive extractions. Ogner and Schnitzer (1970) have found that soil fulvic acids complex phthalate esters and that the phthalates cannot be completely extracted from fulvic acid materials. These findings suggest that the organic solvent extraction procedure used herein may not give quantitative recovery of the fatty acid and phthalate esters. However, the relative percent of each material extracted would be quite high due to the low amounts of fulvic acid materials that are likely present in anaerobically-digested sludges (Terry et al., 1979). Because of the significant amount of phthalate esters detected in all three sludges, the types of phthalate esters found, and the ubiquitous use of these compounds in industry, the evidence indicates that the majority of phthalate esters found in these sludges originate from industrial sources.

No detectable polychlorinated biphenyls (PCB's), polybrominated biphenyls (PBB's), or chlorinated hydrocarbon pesticides were present in any of the three sludges. The detection limit for PCB's and PBB's with the GC-MS system used was about 10 μg/g.

From the MS data, it was noted that essentially no organic compounds were present in any of the three sludges that had molecular weights of less than 200. This finding may be explained through several lines of reasoning. First, since these sludges were collected from sand drying beds, the smaller molecules may have been water soluble and were leached from the sludge. Secondly, low molecular weight compounds, especially those soluble in nonaqueous solvents, tend to possess a high vapor pressure resulting in loss from the sludge through volatilization. In both cases low molecular weight organics would be completely removed from the sludges prior to collection. Thirdly, these molecules may undergo rapid microbial attack resulting in liberation of gaseous products (e.g., CO_2) or incorporation into more complex organic molecules.

Carbohydrates

The total carbohydrate content of the three sludges as determined by each of the three hydrolysis procedures in conjunction with the anthrone colorimetric method for monosaccharides is given in Table 3.5. A positive anthrone reaction is obtained with hexoses, hexuronic acids, and aldopentoses such as xylose (Dische, 1962) which were found to be the only sugars present in the hydrolyzates of the three sludges as determined by HPLC. A 2 hour reflux of sludge with 0.5 M H_2SO_4, yields the highest percentage of recoverable sugars and apparently results in the least amount of carbohydrate destruction. The method of Oades et al. (1970) involving the 26 hour soak with 13 M H_2SO_4 was found to decompose a sizeable amount of carbohydrates. This finding becomes apparent when a comparison of the monosaccharide recovery obtained with the 26 hour soak with 13 M H_2SO_4 is made with the 2 hour reflux with 0.5 M H_2SO_4; a 20 to 33% lower total carbohydrate content was found with the Oades et al. procedure for each of the three sludges. When compared to the 0.5 M H_2SO_4 reflux method, the procedure involving a 30 minutes soak in 11.5 M H_2SO_4 also showed a slight reduction (from 4 to 14%) of total recoverable carbohydrates. From a purely quantitative standpoint, the 2 hour reflux in 0.5 M H_2SO_4 was judged to be the best method for carbohydrate extraction, although the 30 minute soak with 11.5 M H_2SO_4 procedure yielded similar results.

Examination of the spectra obtained from the three procedures revealed that the method of Oades et al. (1970) and the 0.5 M H_2SO_4 reflux procedure produced only carbohydrate oligomers. However, Browning's (Browning, 1967) hydrolysis method (11.5 M H_2SO_4 soak) produced only monomeric carbohydrates that were readily separated by HPLC. The HPLC spectra indicate that the 26 hour soak with 13 M H_2SO_4 and the 0.5 M H_2SO_4 reflux procedures hydrolyzed cellulose and hemicellulose polymers into water soluble oligomers, but the hydrolysis methods did not cleave the oligomers to carbohydrate monomers as was obtained with the 30 minute soak with 11.5 M H_2SO_4. No reasonable explanation can be suggested to explain this finding. The high oligomeric content detected by the 0.5 M H_2SO_4 reflux and the 13 M H_2SO_4 soak procedures indicates that almost all of the carbohydrates found in these sludges were present as either hemicellulose or cellulose because of the relative stability to microbial decomposition of the beta linkages found in hemicellulose and cellulose polymers. Carbohydrates with alpha linkages such as starch would likely be rapidly decomposed during sludge digestion. The only monosaccharides found to be present in sizeable quantities in hydrolyzates from the three sludges were hexuronic acids, xylose, and glucose.

The total hexuronic acid content of each sludge was determined by the carbazole-H_2SO_4 analysis procedure (Table 3.6). The 30 minute soak with 11.5 $\underline{M}$ H_2SO_4 tended to yield higher hexuronic acid contents for the three sludges than other hydrolysis procedures. The carbazole-H_2SO_4 reagent may react more readily with a free hexuronic acid than with hexuronic acid bonded in a carbohydrate oligomer. The 30 minute soak with 11.5 $\underline{M}$ H_2SO_4 would therefore be a better estimate of the actual hexuronic acid content of the sludges than either of the other two procedures.

Because of the relatively high yields of anthrone-reactive substances and the almost complete hydrolysis to monomers, the hydrolyzates obtained by the 30 minute soak with 11.5 $\underline{M}$ H_2SO_4 were used in quantitating the amounts of the major carbohydrates present in each of the sludges. The HPLC analysis of the sludge hydrolyzates established that the only monomers present in sizeable quantities were glucose, xylose, and the hexuronic acids which consisted of either glucuronic acid, galacturonic acid, or some combination of the two. Table 3.7 gives the amounts of monosaccharides found in hydrolyzates of Anderson, Marion, and Frankfort sludges. Glucose was the most abundant monosaccharide present in sludges (concentrations varied from 0.45 o 1.65%), whereas the amounts of xylose and hexuronic acids present varied from one-sixth to one-half that of glucose. Small amounts of glucosamine were also present in sludges (concentration varied from 0.01 to 0.05%). The sum of the weights of all monosaccharides represented 0.99, 1.89, and 2.55% of the oven-dry weight of Anderson, Marion, and Frankfort sludge, respectively. This finding suggests that carbohydrates are a relatively unimportant component of sludges at least when viewed on a weight basis. It seems likely that most of the carbohydrates present in raw wastewater are decomposed during the sludge treatment process.

Glucose monomers in sludge hydrolyzates are believed to have been derived from cellulose polymers, whereas the xylose and hexuronic acids monomers are likely the basic constituents of the hemicellulose polymers. Heukelekian (1930, 1958) has reported that hemicellulose and cellulose materials can be degraded during anaerobic digestion. However, degradation of hemicellulose and cellulose was much slower than that of starch or free sugars because of the beta linkages in the hemicellulose and cellulose polymers. Because of the resistance of these polymers to microbial attack, some of the hemicellulose and cellulose present in wastewater apparently was not decomposed and remained unaltered in the sludge fraction.

The fact that glucosamine was detected in each of the three sludges indicates that some carbohydrate in sludges may be of microbial origin because amino sugar compounds are derived largely from microbial cells walls. Furthermore, the amino sugar analysis procedure used is not specific for glucosamine and other sugars such as muramic acid (3-O-α-carboxyethylglucosamine) may have been included. Muramic acid and galactosamine are present only in bacterial cell walls (Painter et al., 1961) and are likely found in sludges with other amino sugars.

Organic Nitrogen Components

The total organic N content comprised 0.64, 0.59, and 1.52% of the oven-dry weight of the Anderson, Marion, and Frankfort sludges, respectively (Table 3.8). The low organic N values, when compared to previous work (Sommers et al., 1972) likely result from the sludges having been treated on sand filter drying beds where partial aerobic decomposition and mineralization of organic nitrogen occurred and from dilution of sludge organic matter by sand particles. From 97 to 99.2% of the total organic N was accounted for in the combined hydrolyzable- and nonhydrolyzable-N fractions. The reamining 1 to 3% may represent experimental error or the N may be present in the fulvic acid fraction derived from the sludge residue after HCl hydrolysis which was not analyzed for organic N.

Data in Table 3.8 confirm previous work reported by Sommers et al. (1972) which established that α-amino acid N was the predominant form of N present in anaerobically-digested sewage sludges. Alpha amino acid N represented from 31 to 36.5% of the organic N in sludges examined. The NH_4^+-N, which comprised 16.5 to 23.4% of the organic N, originates from easily acid hydrolyzable-N bonded in amide linkages such as the NH_2 groups in the amino acids glutamine and asparagine. Low molecular weight amines of sufficient volatility to be vaporized and subsequently condensed during the steam distillation-acid titration analysis procedure would also appear in this fraction. Hexosamine-N was found to constitute from 1 to 4% of the total organic N present in sludges. This finding is in good agreement with the work of Sommers et al. (1973) who reported from 1 to 3% of the total sludge N as hexosamine-N. Unidentifiable N was the second largest organic N fraction accounting for 30.9 to 34.4% of the organic N. This fraction contains higher molecular weight primary, secondary, and tertiary amine compounds along with other compounds such as purine and pyrimidine bases, phosphoethanolamine, phosphonoethanolamine, and amino acids containing one or more amino groups not in the alpha portion (i.e., arginine, β-alanine, citrulline, γ-amino butyric acid, histidine, lysine, ornithine, and tryptophan).

The nonhydrolyzable N, which comprised 4 to 13.9% of the total organic N, was divided into a NaOH extractable fraction (humic acid-N) and a NaOH insoluble residue and analyzed for total N by the method of Nelson and Sommers (1972). The N in both fractions is thought to exist as either heterocyclic N or as α-amino acid N that has bonded to an aromatic ring via a nitrogen-carbon bond. Both structures would be stable to acid hydrolysis and base extraction. The organic complex to which the N was bonded would determine if the N is humic acid-N or residue-N. Each of the two N fractions account for about one-half of the nonhydrolyzable-N in the Anderson and Marion sludges suggesting that humification of proteinaceous material was occuring to give a stable nitrogenous organic product. The residual-N accounted for one-fifth of the Frankfort sludge nonhydrolyzable-N fraction suggesting that the humification process had not proceeded to the extent observed in the other sludges.

Heukelekian (1958) has reported that the crude protein content of raw sewage increases on a percent ash-free basis as the sewage is converted to digested sludge. Crude protein analysis has suggested that decomposition of the protein fraction did not occur as rapidly as did decomposition of the other sludge constituents. Heukelekian and Balmat (1959) and Kahn and Wayman (1964) have shown that sixteen amino acids were commonly present in raw sewage. All sixteen of these amino acids were found in the sludges examined along with several amino acids of bacterial origin such as α-amino pimelic, α,ε-diaminopimelic, β-alanine, γ-amino butyric, ornithine, cysteic acid, methionine sulfone, and methionine sulfoxide (Table 3.9). The presence of relatively large amounts of amino acids of bacterial origin indicate that each of the sludges has undergone extensive microbial decomposition and that bacterial cell products are sludge components. The data also suggests that the protein fraction of sewage sludge may have decomposed as rapidly as some of the other organic fractions, but that bacterial protein synthesis also occurred to give an apparent slower rate of protein decomposition during sludge digestion. A determination of the α-amino acid N concentration in a sludge sample does not detect this phenomenon, where determination of the amino acid distribution would readily detect coincident protein degradation and synthesis during sludge digestion.

A comparison of the protein content of sludges as estiamted by dividing the α-amino acid N content by 0.13 (some proteins contain 13% α-amino acid N) was made with the total protein content as determined by summation of the amounts of individual amino acids (Table 3.10). The results suggest that the protein content determined by summation of individual amino acids is almost twice that estimated from α-amino acid N. From recovery studies with sludge hydrolyzates, it was determined that Bremner's (1965b) method for determining α-amino acid N recovers greater than 95% of added amino acids suggesting that a defect in the method does not account for the discrepancy in estimated protein contents. However, Bremner's method for fractionating organic N in HCl hydrolyzates results in large amounts of unidentified organic N (Table 3.8). Unidentified N represents from 30.9 to 34.4% of the organic N in the three sludges examined. Data for the individual amino acid content in hydrolyzates suggest that the majority of this unidentified organic N may also be in the form of amino acid N not determined by Bremner's procedure which is specific for α-amino acid N. Even though the protein contents as estimated by the two procedures are well within the same order of magnitude, no definite explanation can be given as to why protein content estimates are not closer together.

Nonhydrolyzable Organic Fraction

Approximately the same amount of humic acid was recovered before and after HF treatment of the Marion sludge, whereas HF treatment of the Anderson and Frankfort sludges resulted in a considerable increase in the amount of humic acid extracted by NaOH. The increased recovery of humic acid from the Anderson and Frankfort sludges probably results in increased amounts of phenolics which may be estimated by the Na-amalgam technique.

The phenolic compounds that were present in the humic acid fraction of each of the three sludges are listed in Table 3.12. The spots present on the thin layer chromatography plates were very faint and barely discernable. This finding indicated that although polymers of phenolic-type materials were present in sludges, the amounts are very small when compared to the total amount of humified organic material present. The quantity of each phenolic compound detected was so small that no quantitative measurements were performed on the compounds.

The phenolic compounds found in sludge humic acids have been reported to be of bacterial as well as of a plant lignin origin (Martin and Haider, 1969, 1971; Piper and Posner, 1972b). Thus, it is almost certain that the humic materials found in sludges were produced in microbial transformation of lignin or synthesis from other organics originating from the raw sewage.

A limited number of organic compounds were obtained by using the Na-amalgam method for humic materials extracted from sludges. Two other unsuccessful attempts to use the Na-amalgam reduction method have previously been reported in the literature (Mendez and Stevenson, 1966; Stevenson and Mendez, 1967). In the work of these investigators, infrared analysis of the ether soluble products from the Na-amalgam reduction of soil humic acids revealed the presence of considerable aliphatic material, suggesting that little or no aromatic materials were recovered by the technique. In this work, analysis by ultraviolet spectroscopy of the ether soluble products obtained from most of the Na-amalgam reductions performed revealed no absorption at wavelengths typical of aromatic compounds. This also indicates that little or no aromatic materials were recovered from the nonhydrolyzable sludge residue by the technique.

Since very few organic compounds were identified by Na-amalgam reduction of the nonhydrolyzable sludge fraction, an attempt was made to characterize the humified sludge organic matter spectroscopically. Infrared spectra were taken of the NaOH-soluble humic material and of the NaOH-insoluble residue for each of the three sludges. The infrared spectra for the two Anderson sludge fractions are given in Figure 3.3. The infrared spectra (not shown) of the Marion and Frankfort sludge fractions were similar to those of the Anderson sludge. The spectra of sludge humic acids were also found to be similar to published spectra of soil humic acids (Flaig et al., 1975; Tan, 1975, 1976, 1977; Theng et al., 1966). A strong absorption band appears in the spectra over a wavelength range from 3700 cm^{-1} to 3100 cm^{-1} indicating the presence of hydroxyl groups vibrating over a wide range of energies. Bellamy (1975) has reported that this frequency range would consist of the OH of aliphatic alcohols (3640-3600 cm^{-1}), phenolic OH (3612-3593 cm^{-1}), the OH of carboxylic acids (3560-3500 cm^{-1}), peroxide OH (3550 cm^{-1}), polymeric H-bonded OH (3400-3200 cm^{-1}), and intramolecular H-bonded OH (3200-2500 cm^{-1}). Two major differences are apparent when the hydroxyl group absorption bands of the humic acid are compared to the corresponding sludge residue. A shift of about 70 cm^{-1} tends to occur when the point of hydroxyl maximum absorption of the sludge residue (~3420 cm^{-1}) is compared to the hydroxyl maximum absorption point of the humic acids (~3350 cm^{-1}). This indicates an increase in the polymeric H-bonded OH in the humic acids when compared to hydroxyl absorption in the sludge residue. A broad flat absorption band at 2600 cm^{-1} found in the humic acids, but not in the residues can be attributed to the OH stretching vibration of chelated carboxyl groups (Eltantawy and Baverez, 1978). Williams and Fleming (1966) have also attributed the band at 2600 cm^{-1} to be intramolecular H-bonding of carboxylic acid gorups. The decreased amounts of intermolecular H-bonding and the absence of intramolecular H-bonding of chelated carboxyl groups indicates a decrease in free carboxylic acid gorups or other hydrophilic OH functional groups in the residual sludge organic matter. This finding would explain why the organic matter in the residue was not NaOH-extractable and why such a relatively high amount of residual organic matter remains with the inorganic fraction.

An absorption band (1100-1000 cm^{-1}) found in the spectra of the residue samples was attributed to Si-O absorptions suggesting that not all of the silicate materials had been destroyed by the HF treatment. A sharp absorption band at 2080 cm^{-1} which was detected only in the Anderson humic acid fraction has been found to be an alkylsilane-type compound (Bellamy, 1975). The Anderson sludge was also the only sample to exhibit some mineral-like crystallinity during X-ray diffraction analysis (Figure 3.4). Thus, it is suspected that these two phenomena are related. A summary of the infrared absorption bands and the wavelength assignments is given in Table 3.13. Duplicate samples of each of the three sludges before and after HF treatment were dry-ashed in a muffle furnace to determine the amounts of mineral matter destroyed by the HF. The results indicate (Table 3.14) that the HF treatment removed varying amounts of mineral matter, but not all of the mineral matter was solubilized.

The X-ray diffraction data obtained for the three sludge samples after treatment with HF indicate the presence of very little or no crystalline inorganic minerals (Figure 3.4). A trace of crystalline inorganic material thought to be a feldspar (Brown, 1961) was detected in the HF-treated Anderson sludge. However, the X-ray diffraction spectra of HF-treated sludges were found to be similar to that of carbon black and humic acids published by Pollack et al. (1971). These authors attributed the broad diffraction peaks found at approximately 3.5 Å and at 2.1 Å to either crystalline aromatic structures or graphite-like layers. No diffraction peak was observed at 7.5 Å indicating that no crystalline aliphatic material was present in HF-treated sludges.

Using X-ray diffraction analysis, Yen, Erdman, and Pollack (1961) have proposed a model for petroleum asphaltenes (Figure 3.5). The X-ray spectra obtained from HF-treated sludge was found to be similar to those of petroleum asphaltenes (Yen et al., 1961) and of humic acids (Pollack et al., 1971). It seems likely that the model proposed by Yen et al. (1961) could also be applied to the humified fraction of anaerobically-digested sewage sludge. The maximum intensity diffraction peaks from the sludge humic material occurred at 3.14 Å rather than at 3.34 Å (the maximum intensity band of powdered graphite). However, the observed distance is still much greater than 1.4 Å, which is the approximate bond length of C=C double bonds (Nyberg, 1961). This finding indicates a slight crowding (~6%) of the repeating units in sludge humic material when compared to the graphite crystalline structure, but that the crowding does not approach covalent bond lengths between two atoms. Nyberg (1961) has reported that hydrogen bonding can markedly reduce interatomic distances (up to 1 Å) between two molecular species. Because of the extensive hydrogen bonding observed in the sludge ifnrared spectra, the reduced interatomic distances observed by X-ray diffraction were attributed to hydrogen bonding between the two planes of molecules. The humified sludge organic material may, therefore, exist as layers of molecules that are held together by a combination of van der Waals forces and hydrogen bonding (Figure 3.5). Griffith and Schnitzer (1975) have shown that Fe or Al hydroxy compounds can form stable complexes with fulvic acids. Either amorphous or crystalline mineral matter may, therefore, serve as the nucleus for these bound layers of organic polymers. The coating of this mineral fraction by humic materials would explain the high ash content observed for the sludges after they had previously been treated with HF.

SUMMARY

The organic C distribution in sludges was assigned according to the following scheme. The total organic C concentration was that determined in the inital sludge samples. The organic solvent soluble organic C content (fats, waxes, etc.) was equal to the organic C content in the initial sludge samples minus the organic C remaining in the residue after the organic solvent extraction procedures. The carbohydrate and protein organic C content was the organic C in sludge remaining after the organic solvent extraction procedure minus the organic C remaining in the residue after the 6 _M_ HCl digestion procedure. The residual or humified organic C was assigned as the total organic C content remaining in the residue after the 6 _M_ HCl digestion procedure. The organic C contents of various sludge fractions have been tabulated in Table 3.15.

Unlike raw sewage in which the major organic constituents are fats and waxes, carbohydrates, and proteins (Heukelekian, 1958), a fourth organic fraction, humified fraction has been found to account for 12 to 35% of the sludge organic C (Table 3.15). This finding is evidence that humification of organic compounds either by microbiological or chemical mechanisms plays a vital role in sludge digestion.

Phthalate esters have been detected in the nonaqueous solvent soluble fraction of sludge along with fatty acid esters. Some question has been raised as to whether the phthalates are synthesized by the microorganisms or whether they result from industrial processes. No definite answer can be given based on this study, however, it is likely that both sources may be responsible for phthalate contamination of sludges.

Analysis of the sludge carbohydrate fraction has shown that only hemicellulose and cellulose polymers remain after anaerobic digestion. None of the free sugars found in raw sewage by Painter _et al_. (1961) were detected in any of the three sludges examined. This finding suggests that the free carbohydrates are one of the first fractions subjected to microbial attack during sewage treatment. Therefore, the free carbohydrate fraction may supply some of the basic constituents for synthesis of the humified sludge organic fraction.

The presence of high amounts of amino acids of bacterial origin in all sludges indicates that sludge organic material is derived partially from microbial cells or products of microbial metabolism. What was once thought to be a slow protein decomposition rate in sludges may have actually been the result of bacterial protein synthesis occurring simultaneously with protein decomposition resulting in an apparent slow protein decomposition rate during sludge digestion. The large amounts of hydrolyzable N and amino acids in sludges suggest that fairly rapid mineralization of sludge organic N should occur after addition to soil. Mineralized N could serve as a source of fertilizer N for plants.

A model for the humified organic fraction has been proposed which is consistent with the results obtained from analysis of the residue remaining after HCl hydrolysis. The data strongly suggest, but do not prove, that the humified organic materials of anaerobically-digested sewage sludge exist as layers of quinone-like or condensed ring-like organic material surrounding amorphous inorganic compounds. Because of the resistance of the humic materials to base extraction, strong acids, Na-amalgam reduction, and to microbial attack, the structure proposed for the humified fraction suggests that a sizeable portion of the sludge mineral fraction would not be involved in chemical or microbial interactions if the sludge were applied to agricultural soils. The humified organic fraction envelope would occlude the trapped mineral fraction, which was found to constitute from 9 to 37% of the initial sludge weight (Table 3.14), until microbial decomposition of the envelope had occurred. In soils, this process would occur over a period of years so these inorganic materials would only slowly become available to chemical or microbial reactions.

Table 3.1. General characteristics of sewage sludges studied.

No.	Sludge city	Solids*	Ash	Org C	Total N	NH_4^+-N	Org N	Org C/ org N	Total P	Total S
		%	------------------			% of oven-dry weight				------------------
					Sludges collected from digester					
1.	Anderson	11.2	61.0	21.2	0.91	0.06	0.85	24.9	1.41	0.68
2.	Crawfordsville	1.5	48.0	26.9	2.77	0.15	2.62	10.3	3.50	1.68
3.	Gary	7.2	49.4	26.4	2.06	0.11	1.95	13.5	1.79	1.62
4.	Kokomo	9.9	52.9	25.9	1.23	0.08	1.15	22.5	2.14	1.03
5.	Lafayette	7.7	56.2	22.5	1.60	0.09	1.51	14.9	1.55	1.55
6.	Lebanon	6.9	49.0	26.0	1.73	0.09	1.64	15.9	1.17	1.45
7.	Peru	4.5	51.9	19.8	3.16	0.17	2.99	6.6	1.85	1.96
8.	Tipton	6.8	59.0	22.9	1.89	0.06	1.83	12.5	2.46	1.59
9.	Warsaw	4.9	53.0	22.3	2.84	0.15	2.69	8.3	3.00	1.98
				Sludges collected from sand filter drying beds						
10.	Anderson	-	70.3	15.1	0.75	0.07	0.68	22.2	1.38	0.56
11.	Frankfort	-	69.8	12.6	1.64	0.14	1.50	8.4	1.89	0.96
12.	Marion	-	62.5	9.4	0.72	0.04	0.69	13.6	0.85	0.50

* Present in liquid sludge before drying.

Table 3.2. Organic constituents extracted from sewage sludges by nonaqueous solvents.

Sludge no.	Volatile solids*	Organic materials extracted by: Hexane	Methanol	Benzene	Acetone	Total+	Mixed solvent‡
	------------	------------	------------	mg/g sludge	------------	------------	------------
1	390	119	11	-	-	130	-
2	520	105	29	-	-	134	-
3	506	92	23	-	-	115	-
4	480	229	23	-	-	252	-
5	438	50	11	-	-	61	-
6	510	56	16	-	-	72	-
7	419	46	19	-	-	65	-
8	410	150	24	-	-	174	-
9	470	55	21	-	-	76	-
10	297	100	10	6	5	121	118
11	302	22	13	9	3	47	36
12	375	19	4	7	6	36	40
Avg.	426	87	17	-	-	106	-

* Determined by loss in weight by heating at 650°C for 2 hours.

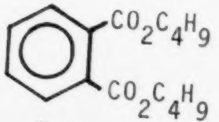
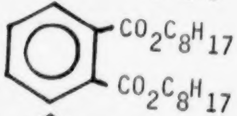
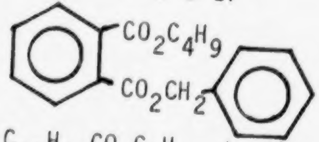
+ Total weight of organic materials extracted by hexane + methanol or hexane + methanol + benzene + acetone.

‡ Mixture of hexane:methyl ethyl ketone:methanol.

Table 3.3. Extraction of organic carbon from sewage sludges by nonaqueous solvents.

Sludge no.	Total organic C	Nonaqueous solvent soluble organic C	Org C extracted by nonaqueous solvents
	-------- mg C/g sludge	--------	% of total
1	201	98	47
2	256	100	39
3	245	81	33
4	241	170	71
5	210	44	21
6	247	64	26
7	188	47	25
8	212	74	35
9	208	56	27
10	151	91	60
11	126	28	22
12	94	24	25
Avg	198	71	36

Table 3.4. Organic compounds present in mixed solvent (hexane:methyl ethyl ketone:methanol) extracts of three Indiana municipal sludges.

Compound	Structural formula	Sludge		
		Anderson	Marion	Frankfort
		--------- % of oven-dry weight⁺ ---------		
Dibutyl phthalate		0.05±0.01	0.006±0.001	0.02±0.01
Di(2-ethylhexyl) phthalate		0.013±0.002	0.006±0.002	0.003±0.001
Butyl benzyl phthalate		ND	ND	0.004±0.001
Wax	$C_{15}H_{31}CO_2C_6H_{13}$ + other fatty acid esters	3.4±0.7	2.7±0.5	3.5±0.6
Wax	$C_{17}H_{35}CO_2C_5H_{11}$ + other fatty acid esters	1.2±0.3	0.5±0.1	< 0.1
Phenolic fatty acid ester	$C_{11}H_{17}CO_2(CH_2)_3COC_6H_4OH$?	7.1±1.1	< 0.1	1.2±0.4
Glyceride fatty acid	Unknown	< 0.1	0.6±0.2	< 0.1

⁺ ND, not detectable

Table 3.5. Comparison of three methods for hydrolysis of carbohydrate material in sludges.

Extraction procedure	Sludge		
	Anderson	Marion	Frankfort
	carbohydrate content (% by weight)		
2.5 *M* H_2SO_4 reflux for 20 min. 13 *M* H_2SO_4 soak for 26 hr. 0.5 *M* H_2SO_4 reflux for 3 hr.	0.60	0.71	2.12
- - - - - - - - - - -			
0.5 *M* H_2SO_4 reflux for 2 hr.	0.90	0.92	2.68
- - - - - - - - - - -			
11.5 *M* H_2SO_4 soak for 30 min. 0.3 *M* H_2SO_4 reflux	0.77	0.88	2.39

Table 3.6. Hexuronic acid content of anaerobically-digested sewage sludges as affected by method of carbohydrate hydrolysis.

Extraction Procedure	Sludge		
	Anderson	Marion	Frankfort
	hexuronic acid content (% by weight)		
2.5 M H_2SO_4 reflux for 20 min.	0.083	0.170	0.422
13 M H_2SO_4 soak for 26 hr.			
0.5 M H_2SO_4 reflux for 3 hr.			
- - - - - - - - - - -			
0.5 M H_2SO_4 reflux for 2 hr.	0.078	0.190	0.618
- - - - - - - - - - -			
11.5 M H_2SO_4 soak for 30 min.	0.265	0.318	0.436
0.3 M H_2SO_4 reflux			

Table 3.7. Monosaccharide content of three anaerobically digested sewage sludges.

Monosaccharide	Sludge		
	Anderson	Marion	Frankfort
	- % of total sludge oven-dry weight -		
Glucose	0.45	1.33	1.65
Xylose	0.26	0.21	0.42
Hexuronic acids	0.27	0.32	0.43
Glucosamine	0.01	0.03	0.05
TOTAL	0.99	1.89	2.55

Table 3.8. Forms of organic nitrogen in anaerobically digested sewage sludges.

Organic N fraction	Sludge		
	Anderson	Marion	Frankfort
	% of total sludge oven-dry weight		
Total organic N	0.64	0.59	1.52
	------ % of total organic N ------		
Total hydrolyzable N	83.2	95.2	89.4
NH_4^+-N	16.5	23.4	19.4
Hexosamine-N	1.3	4.4	3.6
Amino acid-N	31.0	36.5	34.2
Unidentified-N	34.4	30.9	32.2
Total nonhydrolyzable N	13.9	4.0	7.6
NaOH extractable (humic-acid)-N	7.4	2.1	6.1
Residual-N	6.5	1.9	1.5

Table 3.9. Amino acid distribution in three anaerobically-digested sludges.

Amino acid	Sludge		
	Anderson	Marion	Frankfort
	---- µg amino acid/g sludge -----		
amino acids common to animal, bacterial, and plant protein			
Alanine*	403.1	177.9	114.2
Arginine*	679.3	1626.4	3655.6
Aspartic acid*	750.7	212.1	28494.3
Cysteine*	N.D.[+]	175.7	343.2
Glutamic acid*	191.5	139.6	509.9
Glycine*	378.2	175.4	142.8
Histidine*	464.0	409.7	1094.4
Isoleucine*	1258.2	4037.5	3178.7
Leucine*	2170.5	371.6	157.4
Lysine*	580.5	1161.8	4067.4
Methionine	N.D.	822.3	727.6
Phenylalanine*	9117.7	16173.7	4306.4
Proline	37.4	33.5	N.D.
Serine*	195.9	856.7	199.9
Threonine*	67.5	279.9	289.1
Tyrosine*	840.0	1330.0	3196.3
Valine*	2022.1	202.7	1493.1
amino acids of predominantly bacterial origin			
β-alanine	67.3	56.4	103.3
γ-aminobutyric acid	N.D.	15.3	130.7
α-aminopimelic acid	25.8	108.6	238.7
Citrulline	N.D.	N.D.	1918.5
α,ε-diaminopimelic acid	N.D.	1368.7	3641.9
Homocitrulline	N.D.	N.D.	52.4
Ornithine	2866.6	5027.1	8165.1
oxidized forms of amino acids			
Cysteic acid	4.3	1192.3	3994.7
Methionine sulfone	97.3	314.6	11.9
Methionine sulfoxide	312.8	801.9	N.D.

Table 3.9. (Continued)

Amino acid	Sludge		
	Anderson	Marion	Frankfort
	---- µg amino acid/g sludge ----		
other ninhydrin reactive compounds			
Ammonia	213.6	142.2	226.5
Glucosamine	1672.0	N.D.	N.D.
Phosphoethanolamine and/or phosphonoethanolamine	1402.9	1418.0	5194.0
Total	25819.1	38631.6	75648.0

* amino acids detected in raw sewage wastewater.

+ N.D. not detected.

Table 3.10. Protein content of anaerobically-digested sewage sludge as estimated by two methods.

Sludge	Estimation from α-amino acid N	Summation of amino acids
	-- % of sludge oven-dry weight --	
Anderson	1.53	2.58
Marion	1.67	3.86
Frankfort	3.99	7.56

Table 3.11. Amounts of extractable humic acid before and after HF treatment of sludges.

Sludge	Humic acid recovered (% by weight)	
	Before HF	After HF
Anderson	0.67	1.89
Marion	1.73	1.85
Frankfort	0.08	1.30

Table 3.12. Phenolic compounds detected in sludge humic acids.

Compound	Sludge		
	Anderson	Marion	Frankfort
m-cresol	+[*]	+	+
p-cresol	+	+	+
orcinol	N.D.[‡]	N.D.	+
catechol	N.D.	+	N.D.
2,4,5-trihydroxytoluene	N.D.	+	N.D.
p-hydroxybenzoic acid	N.D.	N.D.	+
protocatechuic	N.D.	+	N.D.
p-hydroxycinammic acid	N.D.	N.D.	+

[*] + = detected.

[‡] N.D. = not detected.

Table 3.13. Summary of infrared absorption bands for sludge fractions.

Frequency, cm^{-1}	Assignment	Sludge	
		NaOH soluble material	Residue
3640-3600	aliphatic O-H stretch	m	w
3612-3593	phenolic O-H stretch	s	m
3560-3500	O-H stretch of carboxylic acids	s	m
3550	peroxide O-H stretch	abs	abs
3400-3200	polymeric H-bonded O-H	vs	s
3200-2500	intramolecular H-bonded O-H	vs	w
3050	aromatic C-H stretch	w	abs
2970, 2870	aliphatic methyl C-H stretch	vw	vw
2920, 2850	aliphatic methylene C-H stretch	s	m
1725	nonconjugated C=O stretch	s	sh
1650	conjugated C=O stretch	vs	m
1630	ionized carboxyl group	sh	m
1510	C=C stretch	s	vw
1270-1230	diaryl ethers	vw	abs
1200	aromatic ketone	m	abs
1100-1000	Quartz, SiO_2	vw	m
1050	C-O stretch of polysaccharide	vw	vw

vs, very strong; s, strong; m, medium; w, weak; vw, very weak; abs, absent; sh, shoulder.

Table 3.14. Ash content of sludges before and after exhaustive treatment with HCl-HF.

Conditions	Sludge		
	Anderson	Marion	Frankfort
	Ash content (% by weight) of residue from H_2SO_4 digestion)		
Before HF	92.68	87.54	85.35
After HF	55.14	71.54	21.78
	Ash content (% by weight of initial sludge)		
Before HF	40.50	44.65	34.31
After HF	24.10	36.49	8.76

Table 3.15. Organic C distribution in three municipal sewage sludges.

Carbon fraction	Sludge		
	Anderson	Marion	Frankfort
	% of sludge oven-dry weight		
Total organic C	13.94	9.11	12.66
	% of sludge organic carbon		
Fats, waxes, phthalates, etc.	60.25	24.78	21.60
Carbohydrates and proteins	27.46	40.02	56.13
Humified organic material	12.29	35.20	22.27

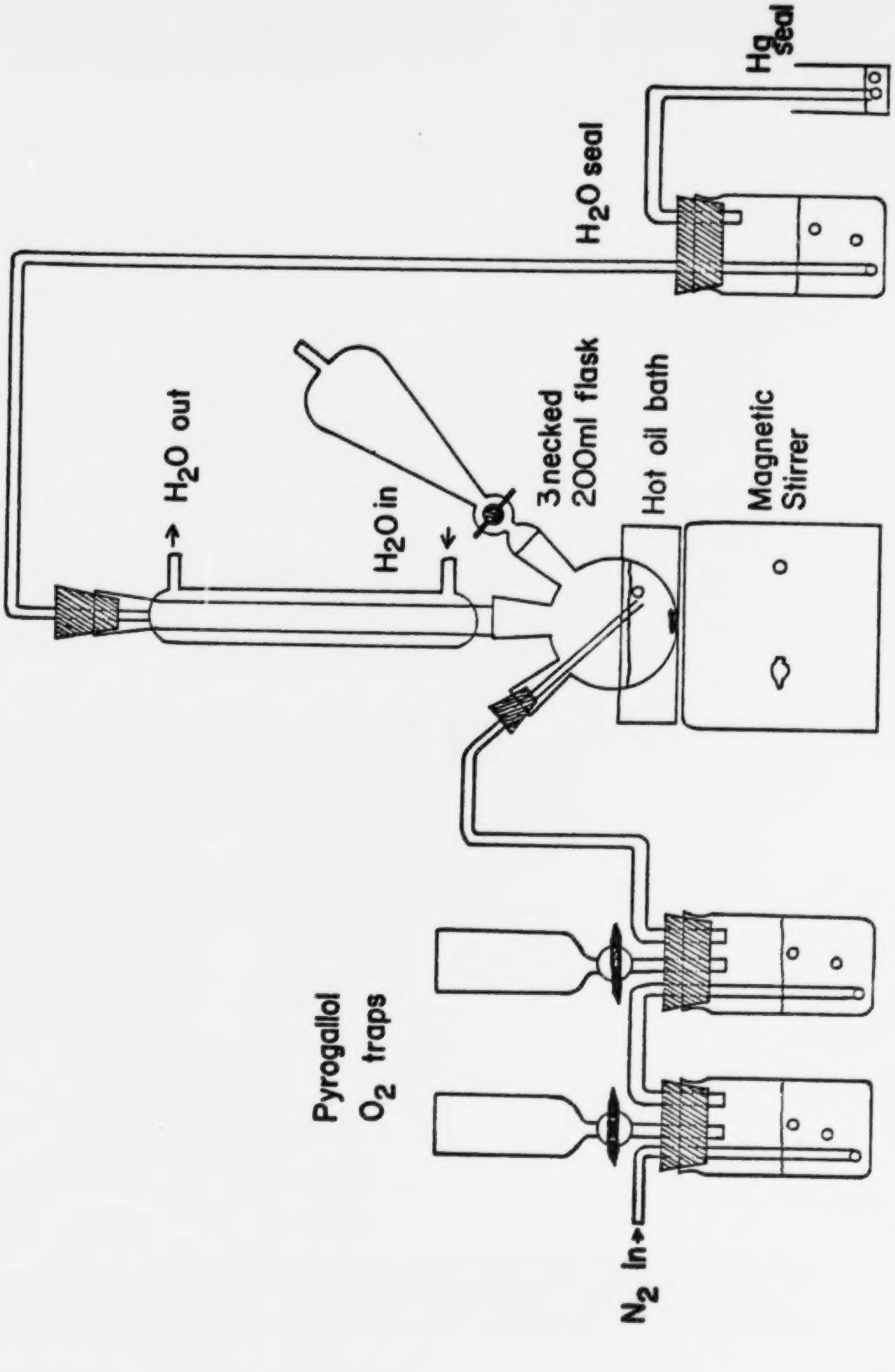

N$_2$ In→
Pyrogallol
O$_2$ traps
H$_2$O in
H$_2$O out
3necked
200ml flask
Hot oil bath
Magnetic
Stirrer
H$_2$O seal
Hg
seal

Figure 3.1. Diagram of the assembled apparatus for the Na-amalgam reduction reaction.

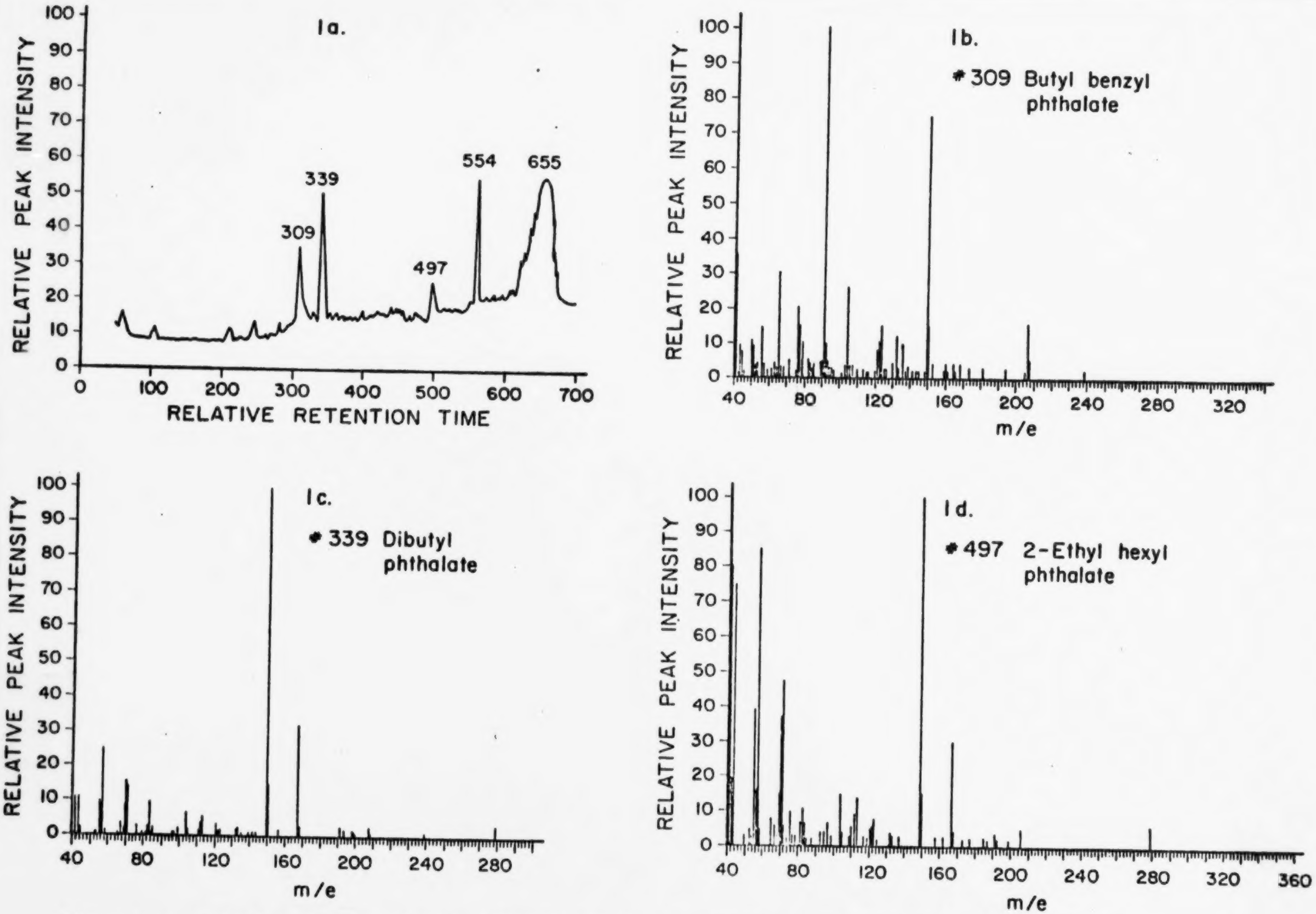


Figure 3.2. Data from GC/MS analysis of the mixed solvent extract of Frankfort sludge. 1a, Total ion current associated with each peak separated by GC; 1b, Mass fragmentation pattern of peak no. 309 (butyl benzyl phthalate); 1c, Mass fragmentation pattern of peak no. 339 (dibutyl phthalate); 1d, Mass fragmentation pattern of peak no. 497 (2 - ethyl hexyl phthalate).

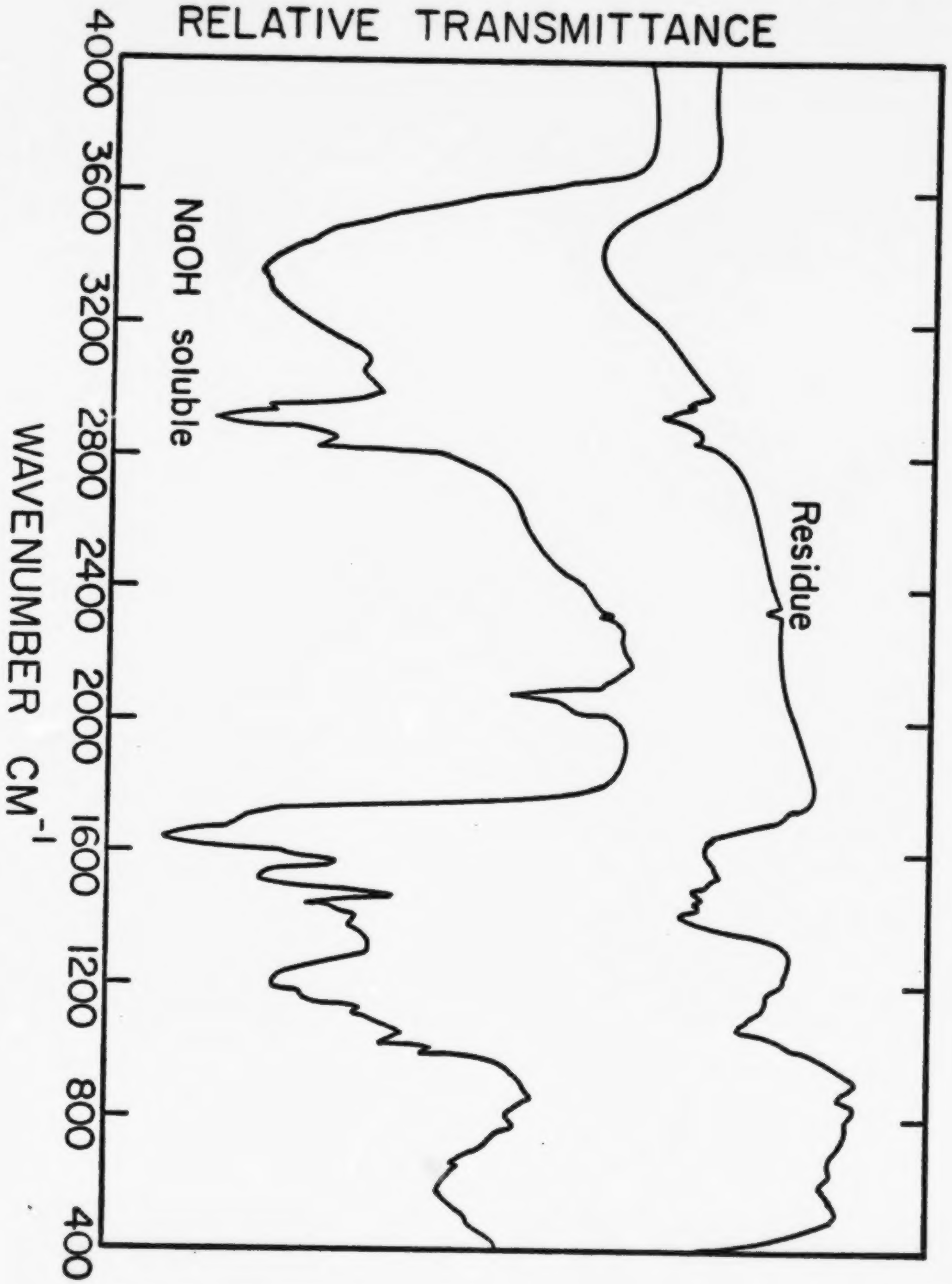


Figure 3.3. Infrared spectra of Anderson sewage sludge fractions.

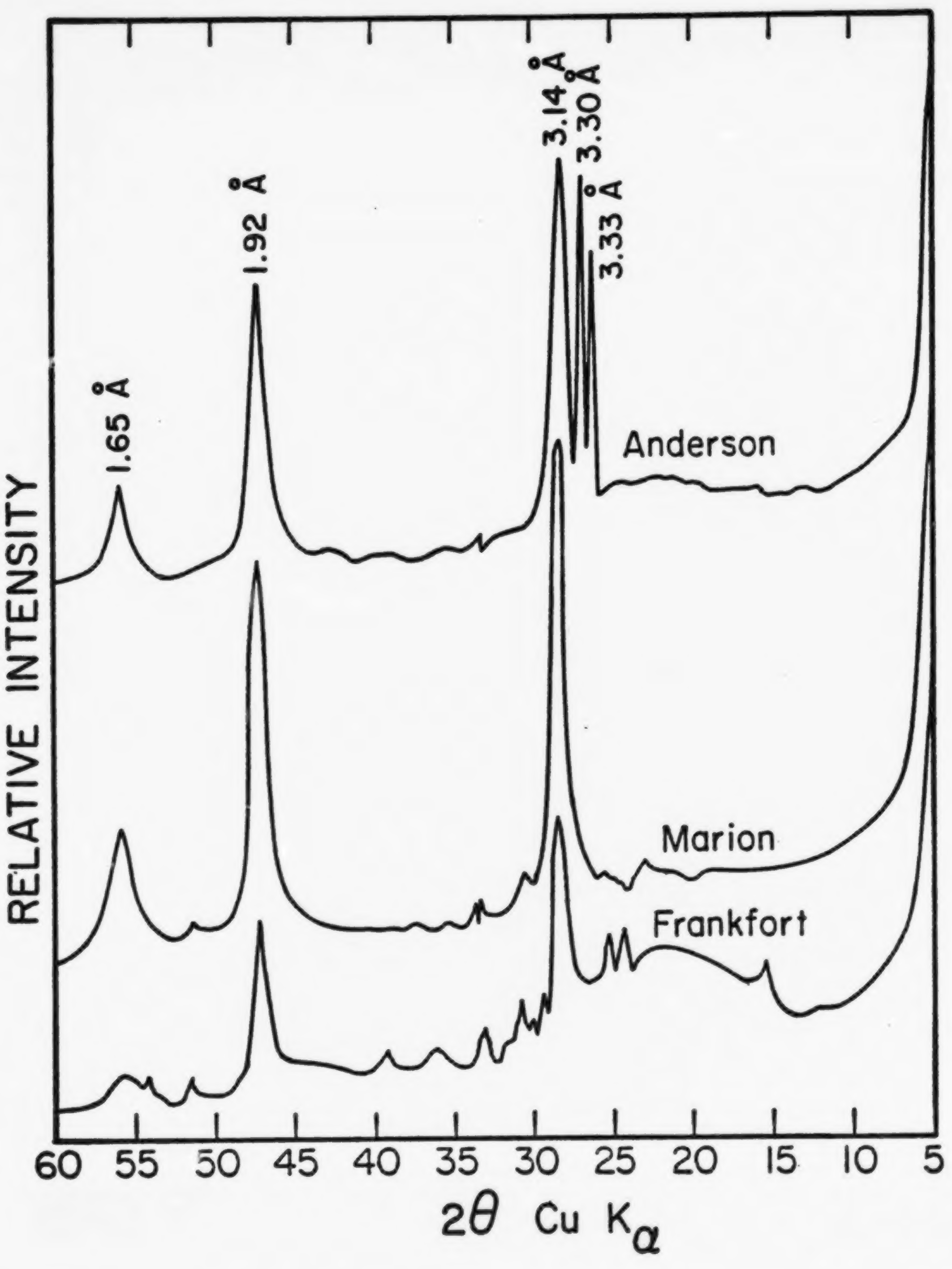


Figure 3.4. Refractometer traces of sludges after HF-HCl treatment.

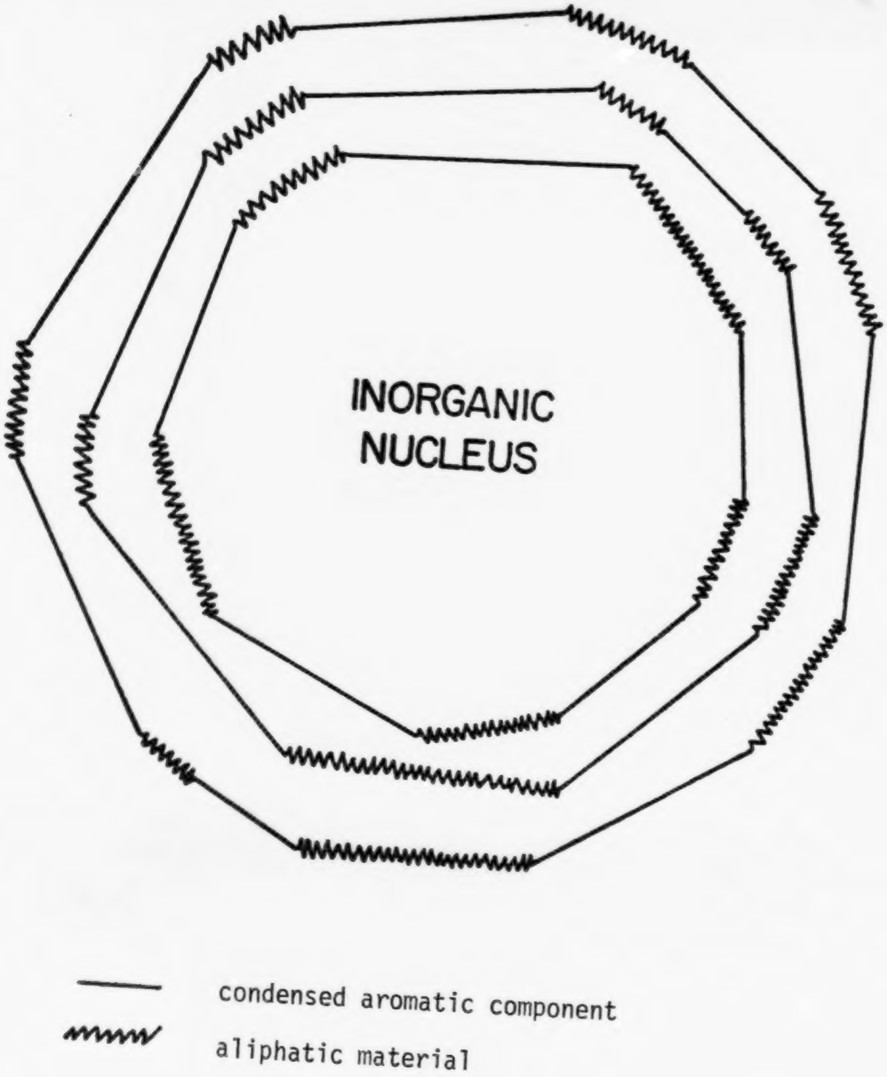

INORGANIC
NUCLEUS
condensed aromatic component
aliphatic material

Figure 3.5. Proposed model for the formation of sludge humified material.

SECTION 4

HEAVY LIQUID SEPARATION OF ORGANIC AND INORGANIC FRACTIONS OF SEWAGE SLUDGE

INTRODUCTION

The recent emphasis of applying sewage sludge on cropland necessitates a knowledge of the constituents contained in sludges and their fate in soils. Sludges contain variable amounts of macro- and micro-nutrients required by plants, as well as non-essential and potentially detrimental trace components (e.g., Cd, Pb, and Ni) (Page, 1974; Sommers, 1977). Previous research indicates that metals in sludge may be present as inorganic forms, such as metal carbonates and/or sulfides (Stover et al., 1976) and also in association with organics (Stover et al., 1976; Sposito et al., 1976; Baham et al., 1978; Holtzclaw et al., 1978; Tan et al., 1971). Because the solubility and subsequent plant availability of metals in soils may vary for inorganic and organic forms, studies of metals in sludges and sludge amended soils would be aided if a technique were available for separating the inorganic and organic fractions present without chemical modification of either fraction.

Organic and inorganic fractions in soils have been separated on the basis of density (Greenland and Ford, 1964; Ford et al., 1969; Ford and Grenland, 1968; Richter et al., 1975). This report describes the application of a heavy liquid technique for separating sewage sludge into organic (light) and inorganic (heavy) fractions.

MATERIALS AND METHODS

Anaerobically digested sludge was obtained from drying beds at sewage treatment plants in Marion, Frankfort and Anderson, Indiana. The sludges were air-dried and ground to pass 60 mesh.

The heavy liquid separation procedure was adapted from Ford et al. (1969) and involved the following steps: five grams of air-dry sludge were weighed into a 50 ml polypropylene centrifuge tube and treated with 20 ml of Fumazone (1,2 dibromo-3-chloropropane; density = 1.7 g/ml; Dow Chemical Corporation) and 0.2 g of sodium dioctyl sulpho-succinate dissolved in two ml of acetone. The sludge-Fumazone-surfactant mixture was sonified for five minutes with a Branson ultrasonic sonifier Model W-350 (20 KHZ power supply). The centrifuge tube was immersed in an ice bath during sonification to maintain a constant temperature. Following sonification the contents of the tube were transferred to a 100 ml polypropylene centrifuge tube using an additional 30 ml of Fumazone to effect quantitative transfer of the sludge. The sludge-Fumazone mixture was then centrifuged for one hour at 2,000 x g and the supernatant, containing the sludge light fraction, was decanted onto a Whatman No. 1 filter paper. The sludge residue was resuspended in 30 ml of Fumazone and the sonification-centrifugation process was repeated twice with the supernatants being combined into a single light fraction. After the third treatment, the sludge residue remaining (i.e., the heavy fraction) was transferred to a Whatman No. 1 filter. The heavy and light fractions collected separately were washed three times with acetone, oven-dried to 105°C for 17 hours, weighed, and ground to < 60 mesh.

The following chemical analyses were performed on the sludge light and heavy fractions: inorganic and total carbon by the Allison (1960) technique modified to collect the CO_2 evolved in NaOH followed by titration to the phenolphthalein end point with standard HCl; organic carbon by difference; NH_4^+ and NO_2^- + NO_3^- were extracted with 2 M KCl followed by steam distillation and acidimetric titration (Bremner, 1965); total N by a modified micro-Kjeldhal procedure (Nelson and Sommers, 1972); organic N by difference; total copper, zinc, cadmium, and nickel were solubilized by a HNO_3 - $HClO_4$ digestion (Sommers and Nelson, 1972) followed by atomic absorption (Varian AA-6 with a hydrogen continuum background corrector); ash content by gravimetric determination after igniting at 650°C for four hours. Volatile solids was calculated from percent ash.

RESULTS AND DISCUSSION

The heavy liquid separation technique enabled separation of the sludge into two fractions with the majority of the sludge weight being present in the heavy fraction (Table 4.1). The heavy fraction of the Mairon, Frankfort and Anderson sludges constituted approximately 88, 88, and 69%, respectively, of the whole sludge weight (Table 4.1). The Anderson sludge had a correspondingly higher percentage of light fraction material. Approximately 8, 4, and 1% of the Anderson, Frankfort, and Marion sludge weights were unaccounted for after the separation procedure (Table 4.1). Much of this weight loss may have been due to solubilization of organic materials during the Fumazone treatments and acetone washings. The low recovery of volatile solids in the Anderson sludge also reflects loss of organic components during the process. The types of compounds not recovered from the Anderson sludge will be discussed in a later section.

Recovery of inorganic C and ash in the fractions was essentially complete with recovery values for inorganic C ranging from 89% to 99% for the Marion and Anderson sludges, respectively, while ash recovery was from 98% to 102% (Table 4.1). The majority of the inorganic C and ash was present in the heavy fraction as shown by the enrichment ratios for inorganic C presented in Table 4.2.

Recovery of organic C in the light and heavy fractions was 87 and 83% for the Marion and Frankfort sludges, respectively, but only 49% in the Anderson sludge. As previously mentioned, the loss of organic materials may have been due to solubilization of non-polar organic compounds (fatty acids, oils, phthalate esters, and waxes) during the fumazone treatments and/or acetone washings. Relatedly, the amount of organic C extracted from sludges by nonaqueous solvents was found to range from 3.6% to 12.1% for the Marion and Anderson sludges, respectively (Section 3). Gas chromatographic-mass spectrometric analysis of the organic compounds extracted from the Frankfort and Anderson sludge revealed that 3.2 to 11.7% of sludge air-dry weight was composed of waxes and fatty acids esters. The relatively high amounts of nonaqueous solvent extractable organic compounds in the Anderson sludge correspond with the relatively high loss of organic material during the heavy liquid procedure. The Marion and Frankfort sludges contained less organic C than the Anderson sludge, resulting in a greater percentage of organic C being recovered in the heavy fraction. However, the organic C content of the heavy fractions was from 50-80 mg C/g as opposed to approximately 300 mg C/g for the light fractions. Thus, the light fractions were enriched in organic C relative to the heavy fractions (Table 4.2).

The light fractions also contained inorganic materials as reflected by the inorganic C and percent ash values (Table 4.1). Infrared analysis of the light fraction has indicated the presence of protein and polysaccaride-like materials, quartz and calcite (Section 5). The presence of quartz and calcite in the light fractions is unexpected since these materials are more dense than Fumazone, suggesting either chemical interactions between sludge organic matter and quartz and calcite or the presence of colloidal particles wherein minerals were coated with sludge organic matter.

The recovery of organic N was greater than that for organic C, but the values were consistent and showed the same trend. From 63-67% of the organic N was found in the heavy fraction of the Frankfort and Marion sludges (Table 4.1). However, all light fractions were enriched in organic N (Table 4.2). In contrast to organic C, organic N was not soluble in acetone or fumazone, which is consistent with the composition of nonaqueous soluble constituents present in the sludges. The ratio of organic C to organic N (C/N) is useful in noting changes in the gross composition of compounds present in sludge fractions (Table 4.2). The C/N ratios of the whole sludge and light fractions of the Marion and Frankfort sludges were similar, whereas, C/N ratio of the Anderson light fraction was lower than the whole sludge. This reflects the loss of organic compounds devoid of N during Fumazone treatments. Heavy fraction C/N ratios were lower than the C/N ratios for whole sludges, indicating that forms of organic N were not the same in the heavy and light fractions.

Recovery of heavy metals in the sludge fractions was essentially complete with values of approximately 100% (Table 4.3). Metal recoveries in excess of 100% were likely due to analytical and sampling errors. Alternatively, metal contamination from the tip of the sonifier probe may have occurred during the ultrasonic dispersion of the sludge samples. The sludge light fractions generally contained a greater concentration of heavy metals than the heavy fraction, as shown by the enrichment ratios in Table 4.2. Incomplete separation of the light fraction from the heavy fraction in the Marion and Frankfort sludges may have caused the heavy fraction to contain a higher percentage of heavy metals than the light fraction. Researchers have found evidence of organic matter-metal associations in sewage sludge (Sposito et al., 1976). Noting shifts in infrared spectra of sludge light fractions following extraction and addition of heavy metals, evidence has been obtained of metal binding sites involving amide N and possibly amide O contained in proteinaceous type materials (Section 5). Some of the metals in the heavy fraction were likely associated with the organic materials bound to the heavy fraction; however, inorganic solid phases of the metals may also be present.

In summary, the heavy liquid separation technique allows the separation of sludge into light and heavy fractions. The light fraction is enriched in organic C, organic N, and heavy metals in comparison to the whole sludge. The heavy fraction contains most of the ash and inorganic C but also contains appreciable amounts of organic matter. The distribution of organic C and N, ash and heavy metals appears to vary for different sludges resulting in differing degrees of separation by the heavy liquid procedure. This procedure provides a means of isolating organic matter in a less altered form that that extracted by the conventional NaOH extraction procedure (Holtzclaw *et al.*, 1976). The heavy liquid separation technique may be useful as an initial step for further characterization of sludge properties and chemistry, particularly in the study of metal-organic reactions.

Table 4.1. Properties of whole sludges and sludge fractions.

Sludge	Fraction	Percent of weight	Ash	Volatile solids	Inorg C	Org C	Org C
		--- % ---	----- % (%*)	------	----------	mg/g (%*)	----------
Anderson	Whole	100	71	29	22	151	6
	Light	25.7	51 (18)	49 (43)	7 (8)	228 (39)	24 (100)
	Heavy	69.3	86 (84)	14 (33)	29 (91)	21 (10)	2 (17)
Marion	Whole	100	63	37	55	94	7
	Light	11.1	39 (7)	61 (18)	5 (1)	305 (36)	22 (36)
	Heavy	88.3	66 (93)	34 (81)	54 (88)	54 (51)	5 (63)
Frankfort	Whole	100	70	30	12	126	15
	Light	8.3	30 (4)	70 (19)	2 (1)	333 (22)	38 (21)
	Heavy	87.8	75 (94)	25 (74)	13 (94)	88 (61)	11 (67)

* Percent of whole sludge

Table 4.2. Ratios for constituents in light and heavy fractions and C/N ratios of sludge fractions.

Parameter	Anderson	Marion	Frankfort
Enrichment ratio*			
Cu	7.5	2.7	1.9
Cd	6.2	1.0	1.4
Zn	8.7	0.9	1.2
Ni	4.0	1.6	2.0
Organic C	10.9	5.6	4.9
Organic N	16.0	4.6	3.3
Volatile solids	3.5	1.8	2.8
Inorganic C	0.2	0.1	0.2
C/N ratio			
Whole sludge	24.4	13.7	8.4
Light fraction	9.4	13.6	8.7
Heavy fraction	13.9	11.1	5.9

* Conc. in light fraction/conc. in heavy fraction

Table 4.3. Heavy metal concentration and recovery in whole sludges and sludge fractions.

Sludge	Metal	Whole sludge	Light fraction		Heavy fraction		Total recovery*
		--- µg/g ---	µg/g	%*	µg/g	%*	--- % ---
Anderson	Cu	4320	14280	85	1910	31	116
	Cd	320	990	80	160	35	115
	Zn	7160	21490	87	2780	27	114
	Ni	2360	6050	66	1530	45	111
Marion	Cu	1550	4150	30	1530	87	117
	Cd	360	380	12	400	98	110
	Zn	5630	5490	11	6350	100	111
	Ni	500	730	16	470	83	99
Frankfort	Cu	1660	3120	16	1620	86	102
	Cd	1590	2370	12	1650	91	103
	Zn	2140	2790	11	2360	97	108
	Ni	900	1670	15	840	82	97

* Percent of whole sludge

SECTION 5

INFRARED SPECTRA OF SEWAGE SLUDGE FRACTIONS EVIDENCE FOR AN AMIDE METAL BINDING SITE

INTRODUCTION

The formation of stable complexes between sludge organic matter and metals is of fundamental importance in determining the mobility and plant availability of sludge borne metals. In order to understand and predict the effect of decomposition of sludge organic matter on metal solubility in soils, a knowledge of metal binding sites is necessary. Although as much as 40 to 60% of sewage sludge consists of organic materials, few studies have been conducted to determine the sites of metal binding in sludge organic matter.

By the use of infrared techniques, humic and fulvic acids extracted from soils have been separated into three spectral types (Stevenson and Goh, 1971). Those belonging to Type I showed equally strong absorptions at 1720 and 1600 cm^{-1}, with no evident absorption in the region of 1640 cm^{-1}. Preparations giving a Type II spectra were distinguished by a strong band at 1720 cm^{-1}, a shoulder at 1650 cm^{-1}, and the absence of a 1600 cm^{-1} band. Type III spectra were characterized by strong absorption bands at 1640 and 1540 cm^{-1} for proteins and a strong band at 1050 cm^{-1} for carbohydrates. The infrared spectra of fulvic acid solutions extracted from sludge-soil mixtures (Sposito et al., 1976) were generally similar to a Type III spectra from soil organic matter. The presence of sulfonic acid groups and functional groups substituted on aromatic rings was suggested in these studies. The results of infrared analysis also suggested, on the basis of a splitting of the COOH band, a complexation reaction between Zn and COO^- groups of the sludge-soil organic matter when a Zn-fulvic acid complex was prepared.

Metal retention measurements of soil organic matter after a variety of chemical treatments revealed a significant decrease in metal retention as a result of blocking acidic carboxyls or phenolic hydroxyls (Schnitzer and Skinner, 1965). Gel filtration studies of trace metal-fulvic acid solutions extracted from sewage sludge, combined with infrared analysis, indicated the presence of proteins and polysaccharides (Baham et al., 1978). Involvement of COOH and SO_3H functional groups in metal-fulvic acid interactions was suggested on the basis of the infrared spectra obtained. Infrared spectra of organic matter extracted from sewage sludge had characteristics similar to those of polysaccharides (Tan et al., 1971). Complexation reactions of Zn with the sludge organic matter resulted in a shift of the OH band from 3500 to 3211 cm^{-1} and an increase in the carboxylate band at 1650 cm^{-1} in the infrared spectra of the Zn complexes. These results suggested the formation of coordinate covalent bonds between OH groups and Zn and electrovalent linkages between COO^- and Zn.

The objectives of this research were to study potential metal binding sites in the organic fractions of sewage sludge isolated by a heavy liquid separation procedure. Metal extraction and addition procedures were used to promote discrete shifts in the infrared frequencies of functional groups associated with metal binding. Secondarily, infrared spectra of light and heavy fractions of sewage sludge were evaluated to yield qualitative information on the major constiuents present.

MATERIALS AND METHODS

Heavy Liquid Separation

Samples of air-dry sewage sludge, obtained from treatment plants in Anderson, Frankfort and Marion, Indiana, were separated into light (organic) and heavy (inorganic) fractions using an ultrasonic dispersion heavy liquid technique (Ford and Greenland, 1968) as described in Section 4. A summary of sludge properties is presented in Table 5.1 and 5.2. Carbon and N were determined by methods described previously (Sommers *et al*., 1976). The sludge and the light and heavy fractions of each sludge were digested in HNO_3-$HClO_4$ and analyzed for metals by atomic absorption using a Varian AA-6 with background correction.

Infrared and X-ray Analysis

Infrared spectra of sludge fractions were recorded with a Perkin-Elmer Model 180 double beam-grating spectrophotometer. Approximately 1 mg of sample and 310 mg of KBr were mixed with a mortar and pestle and transferred to a KBr disc press. Discs (13 mm. diameter) were pressed under vacuum using a total pressure of 7.3 metric tons applied for 5 minutes. Infrared spectra were recorded using a KBr pellet in the reference beam prepared in the same manner as the sample pellet. Coordinates of wave number (cm^{-1}) and percent transmittance were recorded every 0.5 cm^{-1} on cassette tapes with a Hewlett-Packard 2645A terminal using an analog digital converter. All infrared spectral data were subjected to a 25 point mathematical smoothing routine prior to plotting with a X-Y recorder (Savitzky and Golay, 1964; Steiner *et al*., 1972).

Infrared band assignments were made using the following references: (1) Bellamy (1975); (2) Nakamoto (1964); 93) Stevenson and Goh (1971); (4) Nyquist and Kagel (1971); (5) van der Marel and Beutelspacher (1976); (6) Sposito *et al*., (1976); (7) S.S.I.P.S. (1967); and (8) Parker (1971).

X-ray diffraction patterns of the sludge heavy fractions were obtained between 7° and 70° 2θ with a Siemans X-ray diffractometer using Cu radiation $k\alpha$. Samples were affixed to glass slides for x-ray diffraction by wetting with acetone and drying at room temperature.

Sample Treatments

Infrared spectra were recorded of light fractions and of light fractions after (a) extraction with 0.1M EDTA, (b) extraction with 0.1M EDTA followed by treatment with 1M $CuSO_4$, (c) extraction with 1N HCl, (d) hydrolysis with 6N HCl and (e) hydrolysis with 6N HCl followed by extraction with 1M HF. Infrared spectra were recorded of the heavy fractions and the heavy fractions after 6N HCl hydrolysis.

To remove indigenous metals the light fractions were extracted twice for 8 hours with 0.1M EDTA (100:1, V/W) adjusted to pH 6.5. The EDTA extracts were analyzed for metals by atomic absorption using a Varian AA-6. Samples of the EDTA extracted light fractions were shaken with 1M $CuSO_4$ (100:1, V/W) for 24 hours to saturate metal binding sites. Sludge light fractions were shaken with 1N HCl (100:1, V/W) to reduce ionization of carboxylate group. The 1N HCl extracted and EDTA + Cu^{2+} treated light fractions were collected by vacuum filtration, washed with H_2O, washed with acetone and dried for 1 hour at 105°C. The light and heavy fractions were hydrolyzed with 6N HCl under reflux for 16 hours. Silica removal was performed by extracting the 6N HCl hydrolyzed sludge light fractions with 1M HF (200:1, V/W) for 24 hours. The 6N HCl hydrolyzed fractions were collected by vacuum filtration, washed with H_2O, washed with acetone and dried for 1 hour at 105°C. All samples were stored in a desiccator over P_2O_5 before infrared analysis.

RESULTS AND DISCUSSION

The light and heavy fractions accounted for 95.0 to 98.4% of the sludge subjected to heavy liquid separation (Table 5.1). Previous studies (Section 3) of the Anderson, Frankfort and Marion sludges demonstrated that significant amounts of organic C were solubilized by sequential extractions with hexane, benzene, acetone, and methanol using a Soxhlet extractor. The four extracts were combined and analyzed by gas chromatography-mass spectrometry. The major components extracted were waxes and fatty acid esters. These two classes of compounds were the only organic solvent extractable sludge constituents present at concentrations of $> 0.1\%$ of the sludge dry weight. Metals were not present in these fractions. Sludge component recoveries of $< 100\%$ in the present study likely resulted from a portion of the organic solvent extractable fraction being solubilized by Fumazone and acetone used in the separation procedure. Thus, light fractions obtained using the heavy liquid separation procedure contain organic material insoluble in Fumazone and acetone. Additional losses of H_2O soluble and volatile organic materials during the heavy liquid separation procedure may have occurred. However, sludges used in the present study were obtained from sand drying beds where water soluble constituents were leached and volatile components were lost under natural conditions prior to collection of sludge samples. The heavy liquid technique should enable examination of naturally occurring sludge organic-metal complexes because the organics were not exposed to either strongly acidic or alkaline conditions.

IR Spectra of Sludge Light Fractions

Several characteristic absorptions can be seen in the infrared spectra of the sludge light fractions. Major bands occur at 1645, 1530, 1445, 1090, and 1040 cm^{-1} (Fig. 5.1). The light fractions were hydrolyzed with 6N HCl under reflux to eliminate absorption bands due to proteins and polysaccharides (Stevenson and Goh, 1971). Fig. 5.2 shows the result of acid hydrolysis of the Anderson light fraction. Infrared absorptions occurring at 1645, 1530, and 1040 cm^{-1} in the spectrum of the untreated light fraction were eliminated by acid hydrolysis indicating the presence of protein and polysaccharide materials. The major absorption bands of the sludge light fractions are listed in Table 5.3. The carbonyl stretching mode of secondary amides such as those occurring in proteins and polypeptides is referred to as the amide I band and occurs near 1640 cm^{-1} (Bellamy, 1975). Secondary amides also exhibit another characteristic band (amide II) at 1540 cm^{-1} assignable as the N-H deformation mode (Bellamy, 1975). Acid hydrolysis of proteins into their constituent amino acids would eliminate the amide I and amide II bands by breaking the peptide linkages. Similarly, absorptions due to the C-O stretching mode of polysaccharides (strong band near 1050 cm^{-1}) would be eliminated by acid hydrolysis of the polysaccharides into their individual sugar units (Stevenson and Goh, 1971). Thus, the bands (Fig. 5.1) occurring at 1645 and 1530 cm^{-1} are assigned as the amide I and amide II bands and the 1040 cm^{-1} band is assignable as the C-O stretching mode of a polysaccharide.

Quantitative data have been obtained on the carbohydrate and protein content of the sludges studied (Section 3). Carbohydrate content, expressed as a percentage of the total sludge dry weight were: 1%, Anderson; 1.9%, Marion; and 7.2%, Frankfort. Nitrogen present as α-amino N, expressed as a percent of the total sludge dry weight were: 0.19%, Anderson; 0.21%, Marion; and 0.49%, Frankfort. Thus, α-amino N accounted for $\sim$ 28 to 33% of the organic N present (Table 5.1). The protein and carbohydrate data obtained by independent techniques are consistent with the major bands found in the infrared spectra of the sludge light fractions.

A band at 1445 cm^{-1} in the spectrum of the sludge light fractions (Fig. 5.1) has also been eliminated by 6N HCl hydrolysis (Fig. 5.2). This band is assignable as a CO_3^{2-} absorption (Bellamy, 1975) and is due to the presence of $CaCO_3$. The assignment of the carbonate band at 1445 cm^{-1} was confirmed by referring to a standard infrared spectrum of $CaCO_3$ (S.S.I.P.S., 1967) which reveals a second major absorption at 875 cm^{-1}. A band at 875 cm^{-1} in the spectrum of the Anderson light fraction was eliminated along with the 1445 cm^{-1} band by 6N HCl hydrolysis due to dissolution of $CaCO_3$. Identical results were obtained following 6N HCl hydrolysis of the Frankfort and Marion light fractions.

Infrared adsorptions due to aromatic C-H stretching modes (> 3,000 cm^{-1}) were not detected. In addition, the characteristic skeletal stretching mode of the semisaturated carbon-carbon bonds of an aromatic ring near 1600 cm^{-1} (Bellamy, 1975) were not observed in the infrared spectra of the sludge light fractions (Fig. 5.1). This band may have been obscured due to the richness of the spectrum in this region. After 6N HCl hydrolysis the 1600 cm^{-1} region was relatively free of infrared bands, with only weak absorptions occurring at 1705 and 1615 cm^{-1}. Two infrared absorptions near 1700 and 1600 cm^{-1} are characteristically present in the spectra of most humic acid preparations and are enhanced after acid hydrolysis (Stevenson and Goh, 1971). The weak absorptions near 1705 and 1615 cm^{-1} in the infrared spectra of the hydrolyzed sludge light fractions are therefore likely to reflect the maximum contribution of these bands to the spectra of the untreated light fractions. Due to the absence of aromatic C-H stretching vibrations and the very weak intensity of the absorptions near 1705 and 1615 cm^{-1}, there is little evidence for the presence of large quantities of aromatic compounds in the sludge light fractions.

The absorption band at 1730 cm^{-1}, which occurs as a shoulder on the amide I band in the Anderson and Frankfort light fractions and as a band of medium intensity in the Marion light fraction (Fig. 1), was also eliminated by 6N HCl hydrolysis (Fig. 5.2). This band may be due to unionized carboxyl groups of polysaccharides, e.g., uronic acids, (Parker, 1971) or to C=O in the carbonyl groups (Sposito et al., 1976). Absorptions due to the amide N-H stretching mode and the $\overline{OH}$ groups of polysaccharides were partially obscured by a water band in the 3400 cm^{-1} region.

The infrared spectrum obtained after 6N HCl hydrolysis of the sludge light fractions was dominated by the presence of quartz (Fig. 5.2). Even though quartz is more dense tha the heavy liquid used, the presence of quartz in the light fractions may indicate interactions between silica and organic matter. The association of silica with fulvic acids extracted from soil has been demonstrated, however, the nature of this association, whether chemical or physical, was not determined (Griffith and Schnitzer, 1975). All major absorption bands remaining after acid hydrolysis were eliminated by treating the sludge light fractions with HF to dissolve silica. Thus, the very strong band at 1090 cm^{-1}, the weak absorption at 1160 cm^{-1} (shoulder on the 1090 cm^{-1} band), the weak absorptions at 759 and 780 cm^{-1} and the bands of medium intensity at 525 and 470 cm^{-1} are assignable as SiO_2 absorptions. This assignment was confirmed by referring to a standard infrared spectrum of pulverized (< 2 μm) quartz (van der Marel and Beutelspacher, 1976). Again, identical results were obtained for the Frankfort and Marion light fractions.

The two characteristic absorption bands of the ionized carboxyl group occur between 1610 and 1550 cm^{-1} and between 1400 and 1300 cm^{-1} and correspond to the antisymmetrical and symmetrical vibrations of the COO^- structure (Bellamy, 1975). These bands for ionized carboxyls were not detected in the spectra of the sludge light fractions. Although the antisymmetrical vibration of the ionized carboxyl group may be partly overlaid by the amide II absorption (Bellamy, 1975), and a weak absorption near 1400 cm^{-1} was found for the sludge light fractions, these bands were unchanged after treatment of the light fraction with 1N HCl. The addition of 1N HCl to aqueous systems containing the COO^- group reduces ionization and should result in the appearance of carbonyl absorptions in the region of 1740 to 1700 cm^{-1} (Bellamy, 1975). No changes were observed in this region of the infrared spectra of the light fractions indicating a minimal content of carboxyl functional groups in the sludge organic fractions studied.

Sludge Organic Matter - Metal Interactions

The interactions of metals with peptides have been used as models for metal-protein reactions in biological systems. Studies of peptide metal complexes include Cu^{2+} complexes of glycine polypeptides (Hartzell and Gurd, 1969; Bryce et al., 1965). The association of Cu^{2+} with the above peptides was characterized by coordination to the N atom of the peptide bond and concurrent displacement of the peptide bond proton (Gurd and Wilcox, 1956). Studies of metal-protein complexes have employed, to a large extent, Zn^{2+} and Cu^{2+}. Zinc and Cu were bound to serum albumin through a peptide bond carbonyl O and Cu was bound through peptide N (Rao and Lal, 1958). Interactions of Cu^{2+} with metmyoglobin involved coordination to peptide N with displacement of the peptide bond proton (Breslow and Gurd, 1963).

Infrared studies involving the formation of Cu^{2+} complexes of polypeptides have demonstrated the coordination of Cu^{2+} to the amide N of the peptide, glycylglycine (Nakamoto, 1963). In this study of Cu^{2+} glycylglycine complexes, it was shown that the frequency of the infrared absorption band of the peptide carbonyl group (amide I band) decreases as the proton from the peptide N is displaced through metal-N coordination (Kim and Martell, 1964). A smaller decrease in the amide I frequency as a result of metal-N coordination was observed at low pH values (pH = 3.6) without displacement of the proton from the peptide N. At pH values normally encountered in sludge systems (pH ~ 7) the proton from the peptide N would be displaced as a result of metal-N coordiantion.

Infrared data were obtained in an attempt to observe a similar frequency shift of the amide I band due to metal interactions with proteinaceous materials in sewage sludge. Thus, sludge light fractions were extracted with 0.1M EDTA solutions (pH 6.5) to remove indigenous metal cations. Amounts of metals removed by EDTA extractions exceeded 80% of the total metals present in the sludge light fractions for each of the metals measured (Cu, Cd, Pb, Ni and Zn). The infrared spectra of the sludge light fractions after extraction with EDTA showed the following changes from the spectra of the untreated light fractions. (i) The amide I band shifted ~ 11 cm^{-1} from 1644 cm^{-1} in the untreated Anderson light fraction to 1655 cm^{-1} (Fig. 5.3). The Marion sludge showed a similar shift (~ 5 cm^{-1}) of the amide I frequency; however, no shift of the amide I frequency was observed for the Frankfort sludge. (ii) The 1445 cm^{-1} band disappeared after EDTA extraction in each of the sludge light fractions (data not presented). The disappearance of the 1445 cm^{-1} band is due to dissolution of $CaCO_3$ as a result of chelation of Ca^{2+} by EDTA. The shift of the amide I band to higher frequencies in the Anderson and Marion light fractions as a result of metal removal is likely analogous to the shift observed by Kim and Martell (1964) in their glyclyglycine-Cu^{2+} system. To substantiate that the observed shift of the amide I band was due to metal coordination, the EDTA extracted light fractions were treated with $CuSO_4$ to saturate metal binding sites and initiate a shift of the amide I frequency to its original position in the spectra of the untreated sludge light fractions. Fig. 5.3 shows the position of the amide I band for the untreated, EDTA extracted, and Cu^{2+} amended Anderson light fraction. Addition of Cu^{2+} to the EDTA extracted Anderson (and Marion) light fraction broadened the amide I band so that it appeared nearly identical in shape and position to the band observed in the untreated light fraction. In contrast to the Anderson and Marion sludges, an insignificant shift of the amide I band was observed in the Frankfort light fraction after EDTA extraction and Cu addition. The shift of the amide I band to higher frequencies with removal of indigenous metal cations is offered as direct evidence of metal-amide coordination in the sludge light fraction. This shift to higher frequencies was reversed by amending the extracted sludge light fraction with aqueous solutions of $CuSO_4$.

The feasibility of observing a shift of the amide I band as a result of metal extraction can be evaluated by comparing the molar ratios of total metals (Table 5.2) to α-amino N (data given previously). The degree of shift of the amide I band decreased in the same order as the molar ratios of total metals to α-amino N, i.e., Anderson > Marion > Frankfort. The Anderson sludge had the highest molar ratio of total metals to α-amino N ($\sim$ 1.6), followed by Marion ($\sim$ 0.9) and Frankfort ($\sim$ 0.3), and demonstrated the greatest shift in the amide I band ($\sim$ 11 cm^{-1}) after metal extraction. Although the degree of shift of the amide I band cannot be related quantitatively to the amount of protein-metal interaction, the observation of such a shift would be favored as the metal to α-amino N ratio increased.

The peptide-like ligand biuret forms two linkage isomers with Cu^{2+}, the bidentate, O-bonded and the bidentate, N-bonded. Infrared studies of Cu^{2+}-biuret complexes have characterized the carbonyl stretching frequency (amide I band) in each of the linkage isomers. The amide I band was found to be of higher energy in the O-bonded complex. For each of the two isomers there is a lowering of the frequency from that of the ligand, and in the solid state the effect of N-coordination produced a greater decrease (Kedzia et al., 1963). The broad shape of the amide I band in the untreated and Cu^{2+} amended light fraction (Fig. 5.3) may reflect the presence of metal interactions with amide N and O.

The formation of metal N (peptide) or metal O (peptide) bonds is dependent on the metal ion. Cu^{2+} and Ni^{2+}, whose crystal field stabilization energy can be significantly increased by substitution of strong field (N) for weak field (O) donors, would be expected to preferentially bind to amide N, and Zn^{2+} would be expected to bind to amide O (Eichorn, 1973). Additional research is needed to confirm the above mechanism of metal binding in sludges.

Infrared Spectra of Sludge Heavy Fractions

Infrared spectra of the sludge heavy fractions are given in Fig. 5.4, and band assignments are shown in Table 5.4. The infrared spectra of the sludge heavy fractins were dominated by absorption bands of $CaCO_3$ and SiO_2.

Proteinaceous and polysaccharide materials were detected in the sludge heavy fractions as indicated by absorption bands in the 1640 cm^{-1} region (amide I band) and the 1040 cm^{-1} region (C-O stretch of polysaccharide). The infrared spectrum of the anderson heavy fraction after 6N HCl hydrolysis is shown in Fig. 5.5. Hydrolysis with 6N HCl under reflux eliminated the absorption bands at 1640 and 1040 cm^{-1}. All infrared absorption bands assigned to $CaCO_3$ were eliminated by 6N HCl dissolution of $CaCO_3$. Thus, the bands at 2520, 1795, 1435, 875, and 710 cm^{-1} are assignable as $CaCO_3$

absorptions. The position and relative intensity of these bands correspond to those given in the reference spectrum of $CaCO_3$ (S.S.I.P.S., 1967). All major infrared bands present in the sludge heavy fractions after 6N HCl hydrolysis are assignable as SiO_2 absorptions. Thus, absorption bands due to the presence of quartz appear at 1160, 1090, 795, 780, 690, 525, and 470 cm^{-1}. The position and relative intensity of these bands correspond to those given in the reference infrared spectrum of pulverized (< 2 μm) quartz (van der Marel and Beutelspacher, 1976). Caution is advised when interpreting the infrared spectrum of samples containing quartz, as the infrared bands of quartz sharpen considerably when quartz is pulverized to < 2 μm. After pulverizing quartz, it is possibly to view several discrete bands where only a single broad absorption band previously occurred.

X-ray powder diffraction patterns were obtained for the sludge heavy fractions. Comparison of the diffraction patterns obtained to standard powder diffraction data (S.P.D.D.M., 1974) confirmed the presence of quartz and revealed calcite to be the mineral form of $CaCO_3$ present. The primary peaks of feldspar and dolomite also appeared in the sludge heavy fractions as reported previously for other sludges (Silviera *et al*., 1977).

SUMMARY

Light (organic) and heavy (inorganic) fractions were obtained from municipal sewage sludges using a heavy liquid separation technique. Infrared spectra of sludge fractions were dominated by absorptions due to proteins, polysaccharides, calcite and quartz. Infrared analysis of sludge organic fractions indicated a minimal content of carboxyl (COOH) groups and aromatic compounds. An infrared absorption assignable as a C-O stretching vibration was observed indicating the presence of polysaccharides. Infrared absorptions due to the C=O stretching mode (amide I band) and N-H deformation mode (amide II band) were observed indicating the presence of proteinaceous materials.

The position of the amide I band was particularly informative. Direct evidence of metal binding sites involving amide N (and possibly amide O) was obtained by viewing discrete shifts in the position of this band. Extractions to remove indigenous metal cations from the sludge organic fraction resulted in a shift of the amide I band to higher frequencies (~ 11 cm^{-1}). This shift was reversed by amending the extracted sludge light fraction with Cu^{2+}. Metals bound to peptide units in this fashion may be released to the soil solution if the proteinaceous materials in sludge are susceptible to decomposition in soils. Although this study obtained direct evidence of metal binding to amide groups in peptides, the use of infrared spectroscopy does not allow quantitative assessment of the magnitude or percentage of metals bound to sludge organic matter through the mechanism. Furthermore, metals may be bound to sludge organics through other functional groups (e.g., COO^-) but their concentration in the sludge fractions studied was below the detection limit of the infrared techniques used. Nevertheless, the metal binding to peptides demonstrated in this study indicates one fraction of sludge organic matter that deserves further study to characterize the solubility and stability of metals in soils amended with sludge.

Table 5.1. Properties of municipal sewage sludge and sludge fractions.

Sludge	Fraction	Distribution of fractions	Inorg C	Org* C	Inorg N	Org N
		---- % -----	------------------	------------------ %[+]	------------------	------------------
Anderson	Light	25.7	0.7	22.8	0.095	2.255
	Heavy	69.3	2.9	2.1	0.020	0.239
	Whole	--	2.2	15.1	0.074	0.678
Frankfort	Light	8.3	0.2	33.2	0.267	3.808
	Heavy	87.3	1.3	8.7	0.126	1.147
	Whole	--	1.2	12.6	0.144	1.501
Marion	Light	11.1	0.5	30.5	0.061	2.234
	Heavy	87.3	5.4	5.4	0.031	0.487
	Whole	--	5.4	9.4	0.035	0.688

[*] Sum of NH_4^+-, NO_2^--, and NO_3^--N.

[+] % in sludge fraction (oven dry basis).

Table 5.2. Distribution of metals in light and heavy fractions of municipal sewage sludges.

Sludge	Fraction	Cu	Cd	Ni	Zn
		------------------	------------------ μg/g[*]	------------------	------------------
Anderson	Light	14,280	990	6,050	24,190
	Heavy	1,910	160	1,530	2,780
	Whole	4,320	320	2,360	7,160
Frankfort	Light	3,120	2,370	1,670	2,790
	Heavy	1,620	1,650	840	2,360
	Whole	1,660	1,590	900	2,140
Marion	Light	4,150	380	730	5,490
	Heavy	1,530	400	470	6,350
	Whole	1,550	360	500	5,630

[*] μg/g in sludge fraction (oven dry basis).

Table 5.3. Main infrared absorption bands of sludge light fractions.

Frequency cm^{-1}	Assignment	Sludge*		
		Anderson	Frankfort	Marion
2960, 2870	Aliphatic methyl C-H stretch	w	w	w
2920, 2850	Aliphatic methylene C-H stretch	w	s	m
1730	C=O stretch of carboxyl group of polysaccharide or carbonyl groups	sh	sh	m
1645	C=O stretch of secondary amide (amide I band)	s	s	s
1530	N-H deformation of secondary amide (amide II band)	m	m	m
1445	Carbonate, CO_3^{2-}	s	s	s
1380	C-H deformation of C-CH_3 groups		m	w
1090	Quartz, SiO_2	vs	s	s
1040	C-O stretch of polysaccharide	vs	vs	vs
875	Carbonate, CO_3^{2-}	m	w	w
795, 780	Quartz, SiO_2	w	w	w
525, 470	Quartz, SiO_2	m	m	m

* vs, very strong; s, strong; m, medium; w, weak; sh, shoulder.

Table 5.4. Main infrared absorption bands of sludge heavy fractions.

Frequency, cm^{-1}	Assignment	Sludge*		
		Andeson	Frankfort	Marion
2970, 2880	Aliphatic methyl C-H stretch	w	w	w
2930, 2860	Aliphatic methylene C-H stretch	w	w	w
2520, 1795	Carbonate, CO_3^{2-}	w	w	w
1640	C=O stretch of secondary amide (amide I band)	sh	m	sh
1435	Carbonate, CO_3^{2-}	s	m	s
1160	Quartz, SiO_2	sh	w	sh
1090	Quartz, SiO_2	s	s	s
1040	C-O stretch of polysaccharide	s	s	s
875	Carbonate, CO_3^{2-}	m	w	m
795, 780	Quartz, SiO_2	m	w	w
710	Carbonate, CO_3^{2-}	m	w	w
690	Quartz, SiO_2	m	w	
525	Quartz, SiO_2	sh	sh	sh
470	Quartz, SiO_2	s	m	m

* s, strong; m, medium; w, weak; sh, shoulder.

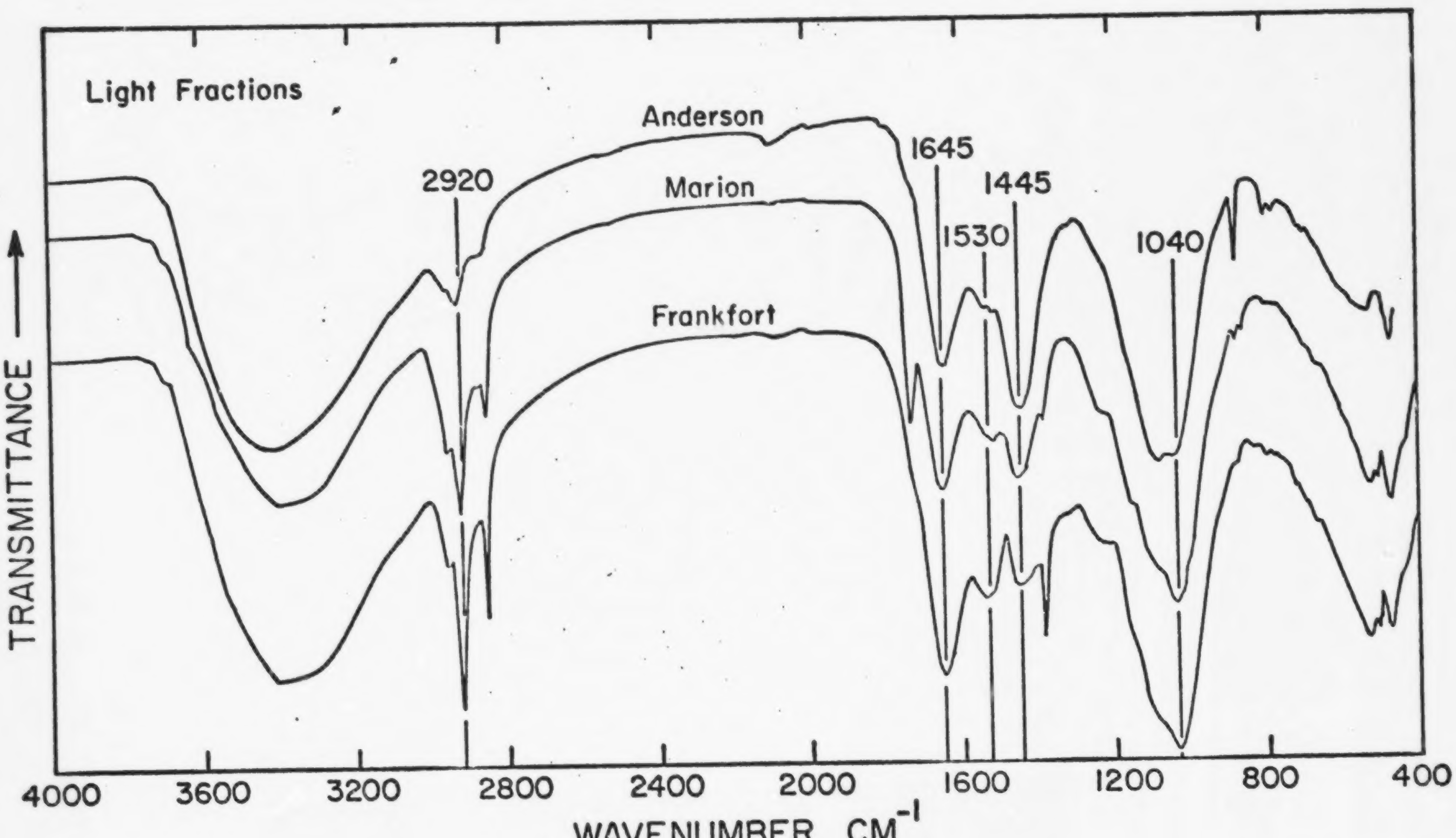


Figure 5.1. Infrared spectra of sewage sludge light fractions.

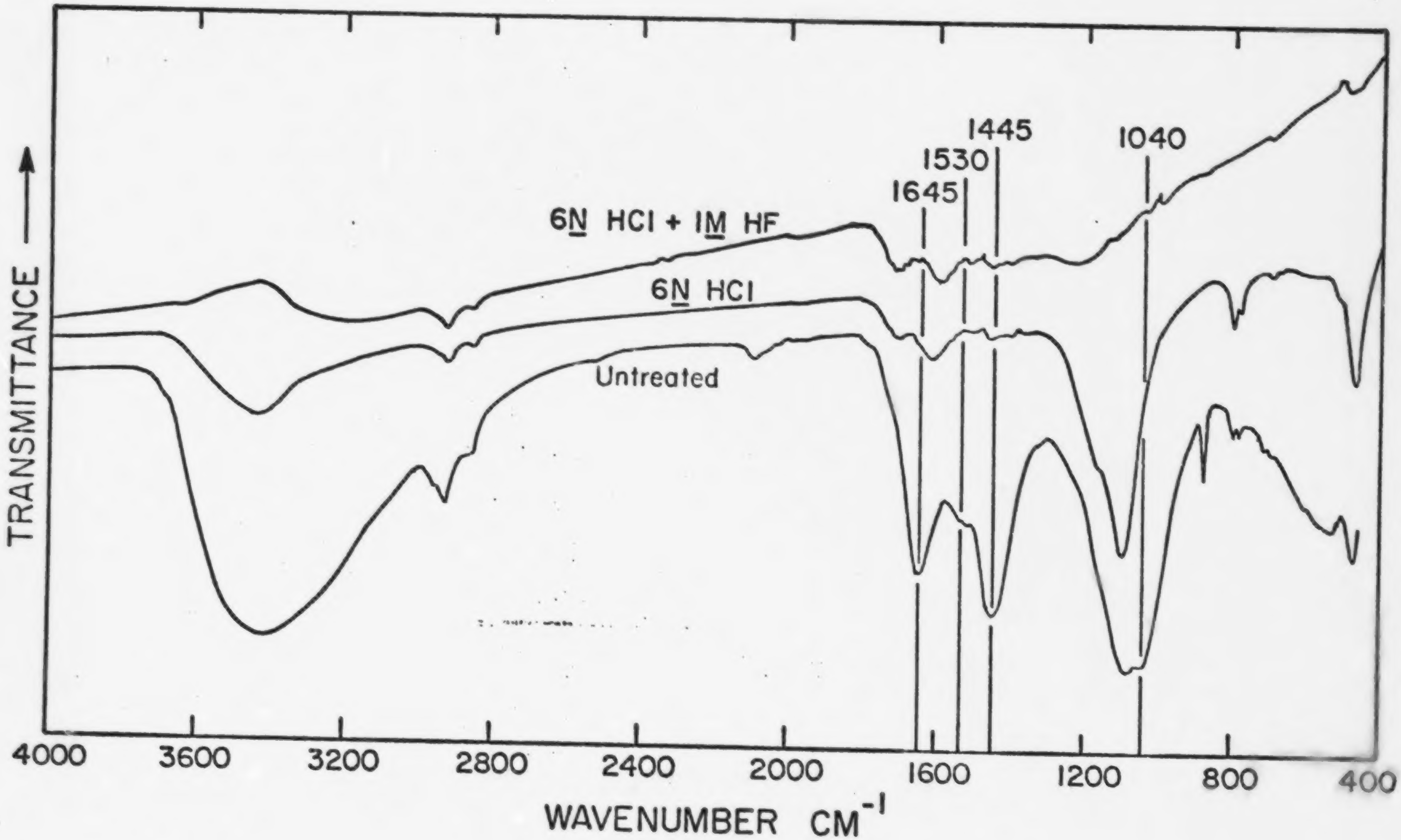


Figure 5.2. Infrared spectra of untreated, 6N HCl hydrolyzed, and 6N HCl hydrolyzed + 1M HF treated Anderson light fraction.

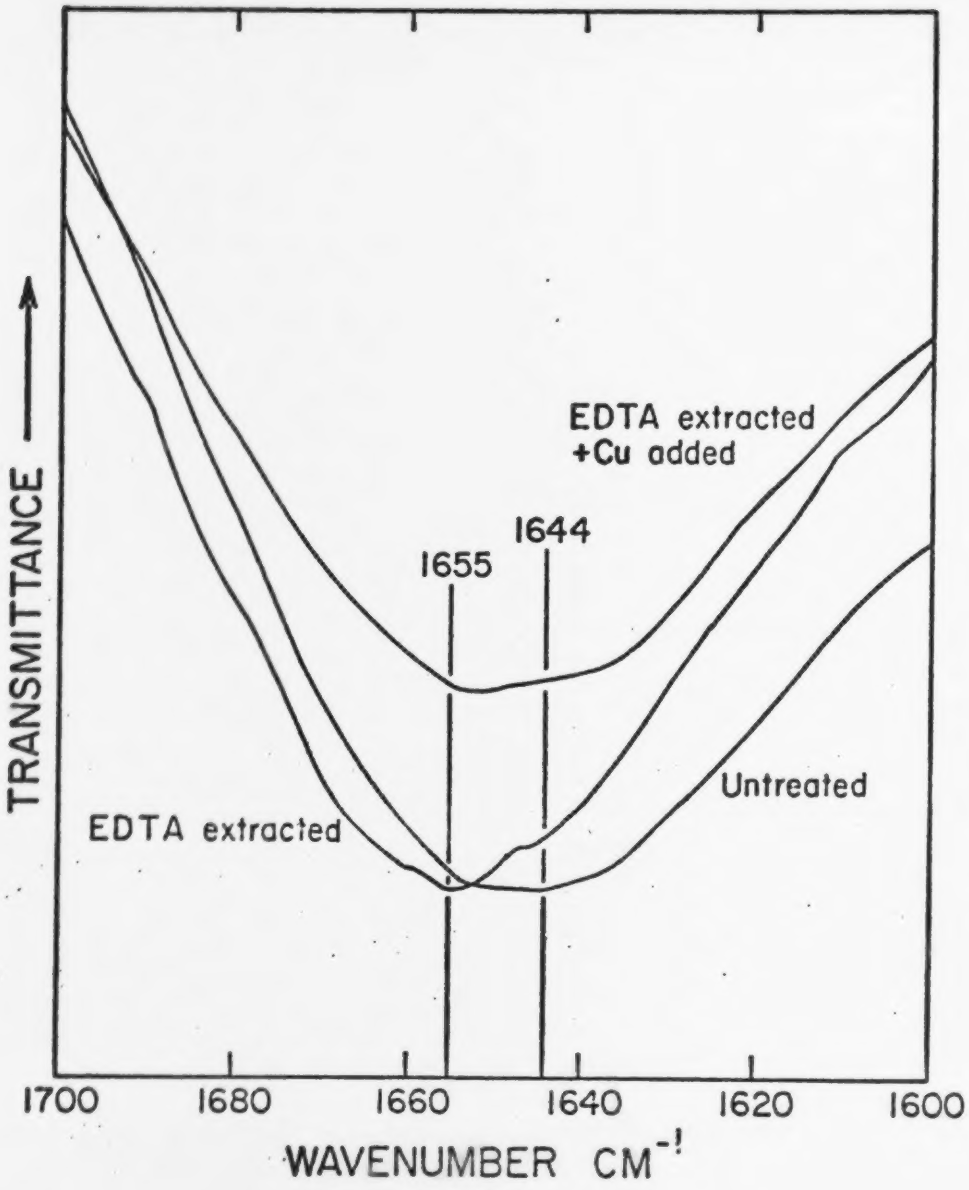


Figure 5.3. Effect of EDTA extraction and Cu^{2+} amendment on the amide-I band of the Anderson light fraction.

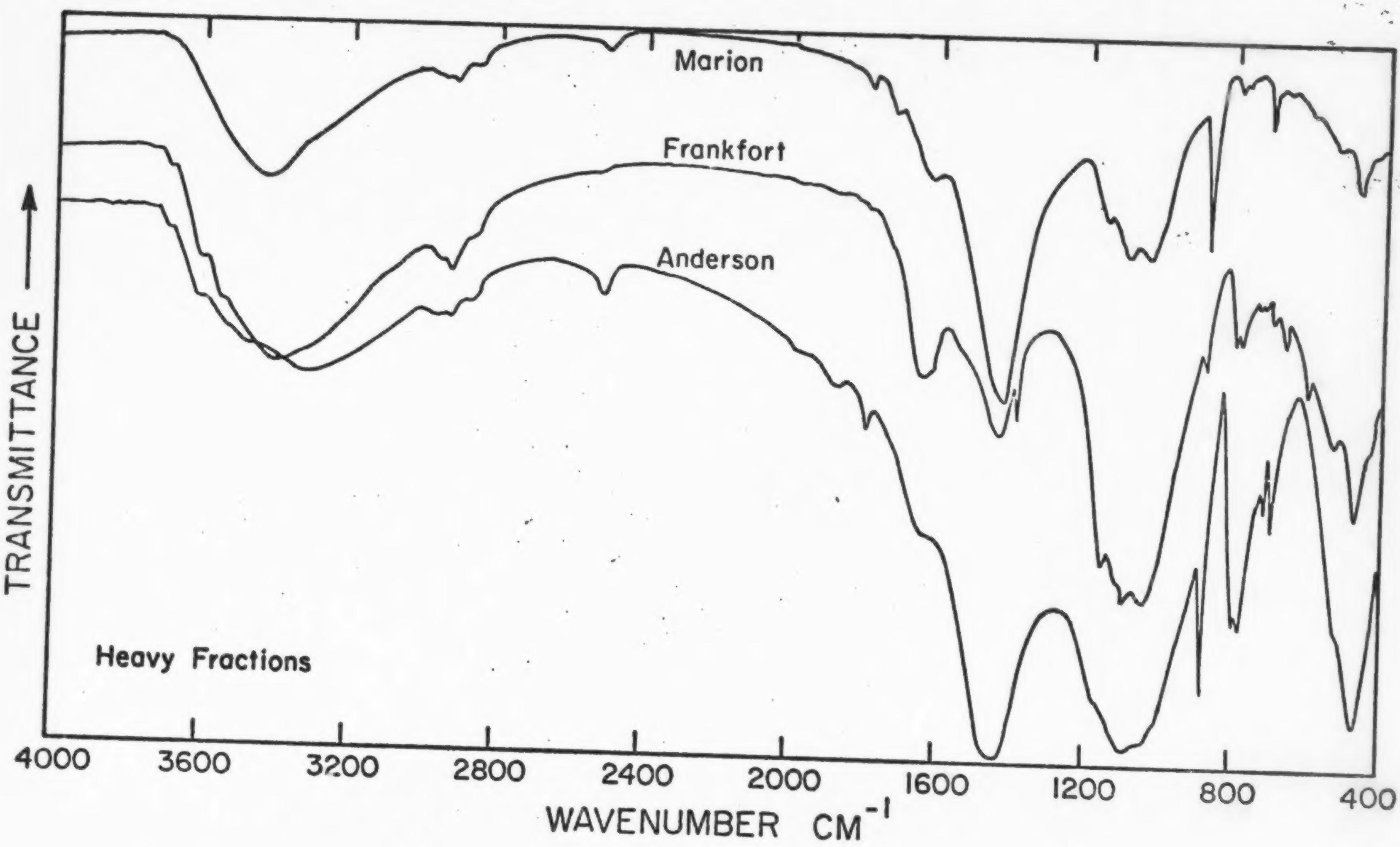


Figure 5.4. Infrared spectra of sewage sludge heavy fractions.

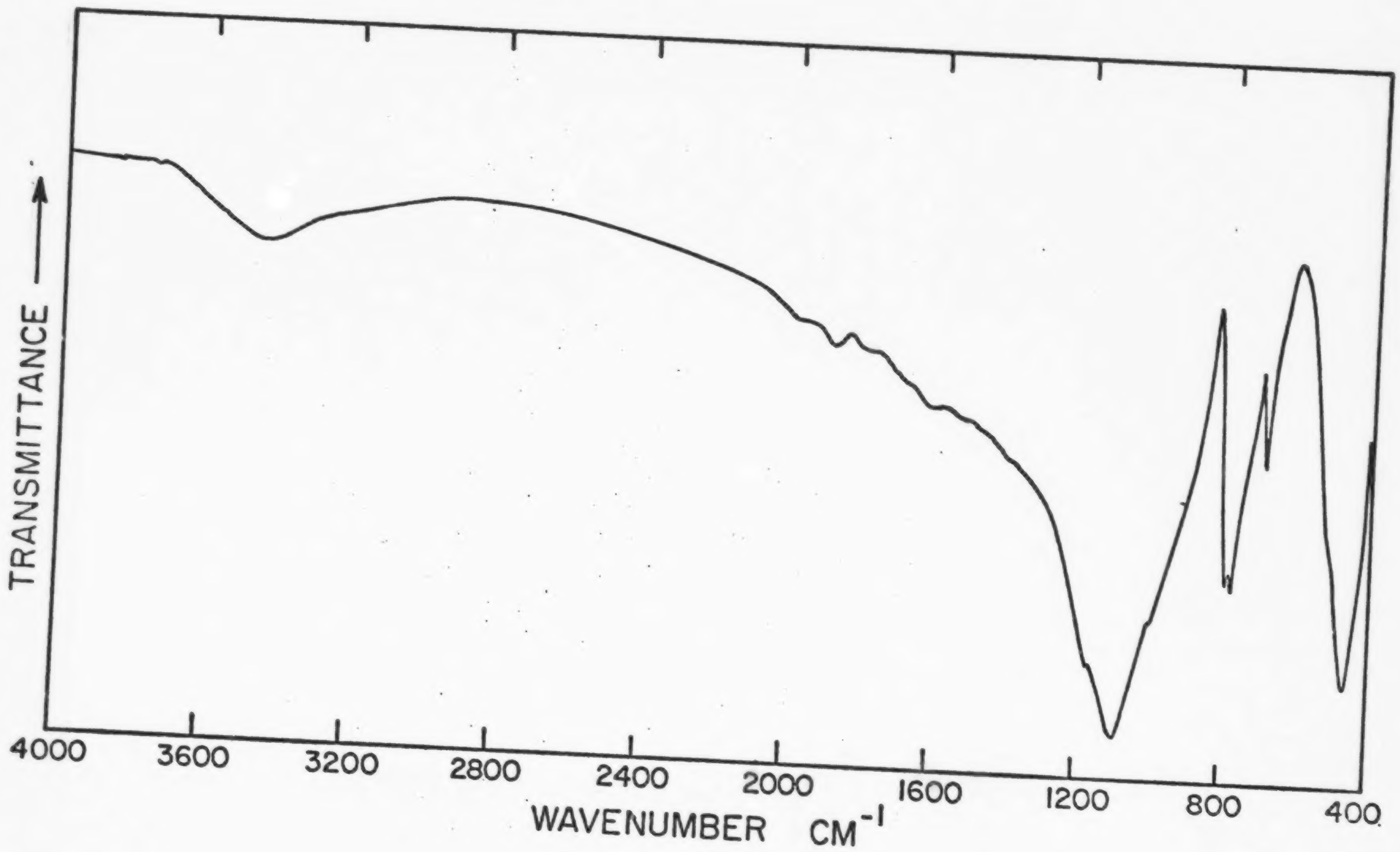


Figure 5.5. Infrared spectra of the 6N HCl hydrolyzed Anderson heavy fraction.

SECTION 6

CHANGES IN THE HUMIC ACID FRACTION OF SOIL RESULTING FROM SLUDGE APPLICATION

INTRODUCTION

The application of sewage sludge to agricultural land is becoming an accepted method of sludge management resulting in the need to understand the fate of sludge constituents in soils. Because sewage sludge is approximately 50% organic matter, its application to soil could affect the status of the soil humic fraction. Several laboratory and field studies have evaluated the decomposition of sludge in soils. Miller (1974) found that sewage sludge was rather resistant to decomposition with a maximum of 20% of the added organic C evolved as CO_2 during a 6 month incubation. In a one year laboratory study of C, N, and metal transformations in soils treated with waste materials, differences in decomposition were observed with different waste materials but soil properties did not influence decomposition (Sommers *et al*., 1979). A field study of sludge decomposition in soil found that the amount of sludge organic C decomposed during a 245 day period ranged from 7 to 21%; values comparable to those obtained in laboratory studies (Section 10). Several studies have shown that the percent of sludge organic C evolved as CO_2 decreased with increasing sludge application rate (Agbim *et al*., 1977; Miller, 1974; Sommers *et al*., 1979).

Carbon cycling during sewage sludge decomposition in soils has been evaluated using ^{14}C labeled synthetic sludge (Terry *et al*., 1979a). This study indicated that the major portion of sludge organic matter was resistant to decomposition during 336 days of incubation. At the end of 168 days of incubation, 62% of the residual sludge organic ^{14}C added was recovered from soils as humic and fulvic acid while only 27% was recoverable in these fractions prior to incubation in soil. Also, the addition of sludge to soil increased the CO_2 released from decomposition of native soil organic matter by approximately 100% over a period of 336 days. In another study using ^{14}C labeled synthetic sludge, it was shown that factors such as soil texture, pH and moisture content have little effect on sludge decomposition rate (Terry *et al*., 1979b). Thus, there is evidence to suggest that a significant amount of sludge organic matter will persist in soils for periods in excess of a year, and that a significant fraction of the sludge organic matter may be recovered in the soil humic and fulvic acid fractions.

In the present study, HA's were extracted from sludge, soil, and sludge-amended soil at varying times after sludge application, and characterized using elemental analysis, functional group determinations, and infrared and visible spectroscopy. The objective of this study was to determine the effect of sludge application on the HA fraction of soil.

MATERIALS AND METHODS

Materials

Soil samples were obtained from field plots established as a contributing project to the W-124 Regional project entitled 'Optimum Utilization of Sewage sludge on Cropland'. The plots were located at the Purdue University Agronomy Farm, W. Lafayette, IN, on a Chalmers silty clay loam (Typic Argiaquoll, fine, mixed, mesic) and consisted of the following treatments in a randomized complete block design: a) no treatment, b) 75 kg N/ha and 10 kg P/ha, and c) 100 metric tons/ha of anaerobically digested and partially dried (50% moisture) sewage sludge obtained from Chicago, IL. The plots were established in May, 1978 and cropped in 1978 and 1979 to barley (*Hordeum vulgare* var. Larker). Fertilizer applications were made each year prior to planting. A single sludge application was made in May, 1978. After barley harvest (approximately July 10), surface soil samples (0 to 20 cm) were obtained from each plot, air-dried and ground to pass a 60-mesh screen prior to analysis. All data presented represent analysis of a composite soil sample for the control and sludge treatments. The composite sample was prepared by mixing equal amounts of all four field replicates. The soil samples were collected at approximately 2 and 14 months after sludge application. The sewage sludge was air-dried and ground prior to analysis.

Extraction of Humic Acid (HA)

Air-dried sludge or soil (100 g) was extracted with 260 ml of 0.1 *N* HCl (to solubilize alkaline earth carbonates) for 3 min followed by filtration under vacuum through a No. 1 Whatman filter paper held on a Buchner funnel. The acid-treated sludge was washed with distilled H_2O until a negative Cl^- test was obtained with $AgNO_3$. A 0.5 *N* NaOH solution was prepared and deoxygenated by purging with purified N_2 for 30 min. The HCl-treated and H_2O-washed sludge was placed in a 1 liter polyethylene bottle and mixed with 500 ml of deoxygenated NaOH. The sludge-NaOH mixture was flushed with N_2, capped, and shaken for 18 hours at 23°C. After 18 hrs, the sludge-NaOH mixture was centrifuged for 20 min at 1850 x *g*. The supernatant was decanted and the solid material discarded. The supernatant was centrifuged at 14,300 x *g* for 20 min to clarify the solution, and the solids discarded. The clarified NaOH extract collected was filtered by vacuum through a No. 42 Whatman filter paper held on a Buchner funnel. To precipitate HA, the filtrate was acidified to pH 5 with concentrated HCl and to pH 1 with 6 *N* HCl added dropwise; pH was measured with a Corning (Model 130) pH meter and a combination glass electrode. The acidified solution containing the HA precipitate was heated to 70°C for 15 min and then held at 23°C for 24 hrs. The solution was then centrifuged for 20 min at 14,300 x *g* to separate the HA precipitate from the fulvic acid. The HA was transferred to a length of Spectrapor membrane tubing (m.w. cutoff: 6,000 - 8,000) with 0.1 *N* HCl and dialized against deionized H_2O for 3 days with the H_2O being changed every 12 hrs. After dialysis, the HA precipitates were centrifuged for 20 min at

14,300 x g. The resulting HA precipitate was lyophilyzed and stored in a dessicator over P_2O_5. The same procedure was used to obtain HA's from the Chalmers soil and sludge-amended Chalmers soil.

Humic Acid Treatments

Portions (0.1 g) of the HA's were hydrolyzed for 16 hrs with 25 ml of 6 N HCl under reflux. The residues were vacuum filtered through a fritted glass funnel of medium porosity, washed with H_2O, lyophilized and stored over P_2O_5 prior to recording ir spectra.

Sodium humates were prepared by adjusting a HA suspension (10 mg HA/10 ml H_2O) to pH 9 with 0.1 N NaOH (Stevenson and Goh, 1971). Samples were air-dried and subsequently stored over P_2O_5.

A portion of the sludge HA was also treated to remove 'loosely held' fatty acids (Schnitzer and Neyroud, 1975). A HA suspension (100 mg HA/25 ml H_2O) contained in a centrifuge tube was placed in a water cooling bath and sonified for 2 hrs using an ultrasonic probe (Branson Sonifier Model W-350, 20 kHZ power supply). One-half of the sonified H_2O-sludge suspension was extracted with hexane by shaking. The resulting emulsion was allowed to stand for several hours and the hexane was separated from the H_2O-sludge suspension using a separatory funnel. The hexane-extracted and the untreated (sonified but not hexane extracted) suspensions were then lyophilized and placed in a vacuum dessicator for 4 hrs prior to recording ir spectra.

Functional Group and Elemental Analyses

Total acidity was determined by the $Ba(OH)_2$ method (Schnitzer and Gupta, 1965). Carboxyl groups were determined by the Ca acetate method (Schnitzer and Gupta, 1965) modified by steam distillating the HA-Ca acetate suspension and titrating the distillate to pH 8.50 with standard base (Holtzclaw and Sposito, 1979). End points were measured using a Corning pH meter (Model 130) with a glass combination electrode. Elemental analysis (C, H, N) was performed by Galbraith Laboratories, Inc., Knoxville, Tennessee, using microcombustion methods.

Spectroscopic Measurements

Optical densities of HA solutions (12.5 mg HA-C/100 ml 0.05 N $NaHCO_3$) were measured at 465 and 665 nm (Chen et al., 1977) on a Beckman ACTA II spectrophotometer. E_4/E_6 values and absorptivities were calculated from these data.

Infrared spectra were recorded with a Perkin-Elmer model 180 double beam-grating spectrophotometer. Approximately 0.7 mg of HA and 310 mg KBr were mixed with a mortar and pestle and transferred to a KBr disc press which was evacuated for 5 min. Discs (13 mm diameter) were pressed under vacuum using a total pressure of 7.3 metric tons applied for 90 sec. Infrared spectra were recorded from 4000 to 250 cm^{-1} with a blank KBr pellet in the reference beam. Coordinates of wavenumber (cm^{-1}) and percent transmittance were recorded in digital format every 0.5 cm^{-1} on cassette tapes with a Hewlett-Packard 2645A terminal. The data were subsequently sent to and processed by the Agricultural Data Network at Purdue University. All infrared spectral data were subjected to a 25 point mathematical smoothing routine prior to plotting with an x-y recorder (Savitzky and Golay, 1964; Steiner et al., 1972). All spectra recorded in percent transmittance were also converted to absorbance to assist in the evaluation of relative peak intensities. The ir spectra in Figure 6.4 were mathematically scaled in absorbance mode such that the amide I bands were of equal intensity.

RESULTS

Elemental and Functional Group Analysis

Results of yield and elemental analysis of C, H, and N for sludge and soil humic acids (HA's) are shown in Table 6.1. Similar yields were obtained for HA separated from soils and sludge; however, when yields are expressed on the basis of percentage of organic C extracted, only 3.6% of the sludge organic C was recovered as HA while 20.1 to 22.8% of the soil organic C was collected as HA. The Chicago sludge HA had the highest N content, over twice that found in the Chalmers soil HA. The ratio of % C to % H (C:H) was highest for the Chalmers soil HA (10.1:1) and lowest for the Chicago sludge HA (7.2:1). Humic acids extracted from the sludge-amended soil had increased N contents and decreased C:H ratios as compared to the Chalmers HA, giving values intermediate between the Chalmers HA and the sludge HA. Specifically, HA's extracted from the sludge-amended Chalmers soil showed the following changes as compared to Chalmers soil HA. (i) Two months after sludge application, N concentration increased by 27.4% and C:H was decreased to 9.5:1; (ii) Fourteen months after sludge application the increase in N concentration was 15.2% and the C:H ratio was 9.8:1.

Results of functional group analysis and parameters obtained from visible spectra of the HA's are shown in Table 6.2. Values of total acidity, CO_2H groups, phenolic OH and absorptivity were highest for the Chalmers soil HA and lowest for the Chicago sludge HA. Humic acids extracted from the sludge-amended Chalmers soil gave values intermediate between the Chalmers HA and the sludge HA. That is, HA's extracted from the sludge-amended Chalmers soil showed the following changes as compared to the Chalmers HA: (i) For HA from the sludge-amended soil 2 months after application, values of total acidity, CO_2H groups, phenolic OH and absorptivity were all decreased, (ii) fourteen months after sludge application, these values were still decreased, but less so. As shown in Table 6.2, E_4/E_6 values were about the same in the HA's extracted from the Chalmers soil and sludge-amended soil. The Chicago sludge had the lowest E_4/E_6 values.

Infrared Spectra

Infrared spectra of the HA's are shown in Fig. 6.1. Specific ir band assignments are listed in Tables 6.3 and 6.4. Infrared spectra were obtained foR the 6 N HCl hydrolyzed HA's (Fig. 6.2) and the Na-humates (Fig.

6.3) to provide additional information regarding the assignment of specific absorption bands.

Chicago sludge humic acid. The ir spectrum of the sludge HA (Fig. 6.1) closely resembled those of recent lake sediment HA's (Otsuki and Hanya, 1967). The sludge HA spectrum was dominated by absorption bands of the amide group at 1655 cm^{-1} (amide I) and 1520 cm^{-1} (amide II). Hydrolysis of the sludge HA with 6 N HCl under reflux, a procedure selected to remove proteins and polysaccharides (Stevenson and Goh, 1971), eliminated the amide I and amide II bands (Fig. 6.2), supporting the above assignment. The possible assignment of the 1655 cm^{-1} band as the C=O stretching vibration of conjugated and/or H-bonded carboxyl groups, was ruled out by examining the ir spectra of the sludge Na-humate (Fig. 6.3). When the carboxyl group is converted to a Na salt, the carboxyl C=O absorption is replaced by two bands near 1600 and 1400 cm^{-1} corresponding to the antisymmetrical and symmetrical vibrations of the carboxylate anion (Bellamy, 1975). The ir spectrum of the sludge Na-humate (Fig. 6.3) showed that the 1655 cm^{-1} absorption was unchanged, confirming its assignment as the amide carbonyl stretching frequency (amide I). An absorption at 1720 cm^{-1}, which occurred as a shoulder on the 1655 cm^{-1} band in the spectrum of the sludge HA (Fig. 6.1), was eliminated in the spectrum of the sludge Na-humate (Fig. 6.3). Concurrently, some increase in absorption occurred at 1600 and 1400 cm^{-1} indicating that the 1720 cm^{-1} band originated from carboxyl groups.

An absorption band at 3200 cm^{-1} occurred as a shoulder on a broad absorption at 3370 cm^{-1} in the ir spectrum of the sludge HA (Fig. 6.1). Due to the predominance of the amide I and II bands in this spectrum, and the high N content of the sludge HA, the 3200 cm^{-1} band was assigned as a N-H stretching frequency of a H-bonded secondary amide (Bellamy, 1975). Secondary amides also exhibit an absorption near 3080 cm^{-1} which is associated with N-H modes (Bellamy, 1975). Aromatic C-H stretching modes also occur in this region. One or both of these modes likely accounted for the absorption band at 3075 cm^{-1} observed in the sludge HA (and the sludge-amended soil HA and soil HA).

A band of medium intensity occurred at 1230 cm^{-1} in the ir spectrum of the sludge HA (Fig. 6.1). This band likely resulted from a combination of the C-O stretch or O-H deformation of a carboxylic acid and the amide III band (Bellamy, 1975). Aryl ethers also absorb in this region. The intensity of this band was reduced in the acid hydrolyzed spectrum (Fig. 6.2) probably as a result of removing the contribution of the amide III absorption. The intensity of the 1230 cm^{-1} band was reduced in the spectrum of the Na-humate (Fig. 6.3) but less so than in the spectrum of the acid hydrolyzed sample, indicating that the amide III band was the primary contributor to the 1230 cm^{-1} absrption.

A band at 1040 cm^{-1} also occurred in the ir spectra of the sludge HA (Fig. 6.1), which was eliminated by acid hydrolysis (Fig. 6.2). This band was assigned as the C-O stretching vibration of polysaccharides (Stevenson and Goh, 1971; Sposito et al., 1976).

A prominent feature in the ir spectrum of the sludge HA was the strong intensity of the bands in the 2850 to 2950 cm^{-1} region (Fig. 6.1). These bands are assignable as methylene C-H (2928, 2855 cm^{-1}) and methyl C-H (2957, 2875 cm^{-1}) stretching vibrations (Bellamy, 1975).

Chalmers soil humic acid. The ir spectrum of the Chalmers HA (Fig. 6.1) resembled a Type III spectrum as labeled by Stevenson and Goh (1971). Specific band assignments are listed in Table 6.4. Major absorption bands occurred at 1718 cm^{-1} and between 1655 and 1620 cm^{-1}. The 1718 cm^{-1} band was assigned as the carboxyl C=O stretching vibration. The major contribution to the absorption in the 1655 to 1620 cm^{-1} region appeared to be the C=O stretching vibration of H-bonded carboxyls (Filip et al., 1974). Analysis of the ir spectrum of the Chalmers Na-Humate (Fig. 6.3) assisted in the assignment of these bands. In the spectrum of the Na-humate, the absorption at 1718 cm^{-1} had been entirely replaced and the 1655-1620 cm^{-1} absorption had been largely replaced by carboxylate absorptions at 1600 and 1385 cm^{-1} (Bellamy, 1975). Thus, in the ir spectrum of the Chalmers HA, the 1718 cm^{-1} band was assigned as the carboxyl C=O stretching vibration and the 1650 to 1620 cm^{-1} absorption was assigned as H-bonded carboxyl stretching modes. In the ir spectrum of the Na-humate a band at 1657 cm^{-1} occurred as a shoulder on the 1600 cm^{-1} absorption, indicating the presence of the carbonyl group. Thus, the 1655 cm^{-1} portion of the 1655 to 1620 cm^{-1} absorption in the ir spectrum of Chalmers HA was assignable as the amide carbonyl stretching frequency (amide I). The spectrum of the acid hydrolyzed Chalmers HA (Fig. 2) was consistent with these assignments. After 6 N HCl hydrolysis, major bands occurred at 1718 and 1606 cm^{-1}. The intensification of the absorption at 1718 cm^{-1} likely resulted from the disruption of H-bonding to carboxyl groups due to acid hydrolysis. This had the effect of shifting the H-bonded carboxyl absorption at 1650-1620 cm^{-1} to the position of the free carboxyl at 1718 cm^{-1} (Filip et al., 1974). The 1655 cm^{-1} absorption (amide I) was eliminated along with an absorption band at 1040 cm^{-1} after acid hydrolysis. The 1040 cm^{-1} band was assigned as the C-O stretching vibration of polysaccharides (Stevenson and Goh, 1971; Sposito et al., 1976). The assignment of the 1606 cm^{-1} band in the spectrum of the acid hydrolyzed HA was somewhat speculative, as had previusly been the case (Stevenson and Goh, 1971). This band was tentatively assigned as an aromatic C=C vibration. Bellamy (1975) has pointed out that the aromatic band at 1600 cm^{-1} is subject to wide fluctuations in intensity. Although the situation is complex, changes in position or nature of substituents on the aromatic ring, such as acid hydrolysis might cause, directly affects the intensity of the 1600 cm^{-1} band. It is possible that the 1600 cm^{-1} band was present at reduced intensity in the ir spectra of the chalmers ha and was obscured due to the proximity of the carboxyl absorptions. After acid hydrolysis, the 1600 cm^{-1} band may have intensified and was no longer buried under the carboxyl absorptions, accounting for its appearance in the spectrum of the hydrolyzed sample.

The 1230 cm^{-1} band, which was stable to acid hydrolysis (Fig. 6.2), but was greatly reduced in the ir spectrum of the Na-humate (Fig. 6.3) was assigned as the C-O stretch or O-H deformation of carboxylic acids (Bellamy, 1975). Amide groups (amide III) and aryl ethers also absorb in this region.

A rather broad absorption occurred in the 1400 cm^{-1} region of the Chalmers HA. This band was assigned as the O-H deformation and C-O stretch of phenolic OH (Stevenson and Goh, 1971). It is noteworthy that this band was absent in the ir spectrum of the Chicago sludge HA, which had the lowest phenolic OH content of the HA's studied (Table 6.2).

Sludge-amended Chalmers soil humic acids. The IR spectra of HA's extracted from the sludge-amended Chalmers soil 2 and 14 months after sludge applicaion are shown in Fig. 6.1. Specific band assignments are listed in Table 6.4. Analysis of these ir spectra was identical to that of the Chalmers soil HA (above) and will not be repeated. Several important trends observed in the ir spectra of the HA's extracted from the sludge-amended Chalmers soil should be noted. (i) The intensity of the amide I band at 1655 cm^{-1} (and less noticeably, the amide II band) was obviously enhanced as compared to the Chalmers HA (Fig. 6.1). The greatest increase in the amide I absorption was observed in the sludge-amended soil HA extracted 2 months after sludge application. The emergence of the amide I band was confirmed in the ir spectra of the Na-humates obtained from the sludge-amended soil (Fig. 6.3). Fourteen months after sludge application the amide I absorption was still increased but less so. (ii) The same trend was observed for the C-H vibrations, with the greatest increase observed in HA extracted from the sludge amended soil 2 months after application and less so for the HA extracted 14 months after application. (iii) Although more difficult to observe due to the intrinsic weakness of the band, there appears to be a similar, but opposite, trend for the band at 1400 cm^{-1}, which orresponds to O-H deformation and C-O stretch of phenolic OH (Stevenson and Goh, 1971). That is, the Chalmers HA gave the most intense 1400 cm^{-1} band which was decreased in HA extracted 2 months after sludge application and decreased less in HA extracted 14 months after sludge application.

DISCUSSION

Elemental analysis and functional group content of an average HA (from means of all data) have been reported (Schnitzer, 1978). This average HA had a N content of 4.7% and a C:H ratio of 11.96:1. The Chalmers soil HA, with a N content of 3.94% and a C:H ratio of 10.1:1 (Table 6.1), closely resembled the average HA. In comparison, the Chicago sludge HA had a higher N content and a lower C:H ratio (Table 6.1). The functional group content of the Chalmers HA (Table 6.2) also compared favorably with that of the average HA (values of total acidity, CO_2H groups, and phenolic OH were 6.7, 3.6, and 3.9 meq/g HA for the average HA, Schnitzer, 1978). In comparison, the sludge HA gave lower values for total acidity, CO_2H groups, and phenolic OH. Based on elemental composition and functional group content, the sludge HA appeared to be a more aliphatic, N rich polymer with lower amounts of carboxyl groups and phenolic hydroxyls as compared to the Chalmers soil and average HA's.

The absorptivity (Table 6.2) of the sludge HA was 1.1 liters g^{-1} cm^{-1} and the absorptivity of the more darkly colored Chalmers HA was 4.7 liters g^{-1} cm^{-1}. The light absorption of humic substances has been shown to increase (higher absorptivity) with increasing degree of condensation of aromatic rings (Kononova, 1966) and the ratio of C in aromatic nuclei to C in alphatic chains (Kasatochkin et al., 1964). Thus, the lower absorptivity of the sludge HA as compared to the Chalmers HA supported the characterization of the sludge HA as a more aliphatic structure.

The ir spectrum of the sludge HA (Fig. 6.1) also supported the above characterization of the Chicago sludge HA. That is, the ir spectrum of the sludge HA was dominated by absorption bands due to amide and methylene C-H groups, while absorption bands characteristic of carboxyls (at 1720 and 1630 cm^{-1}) and phenolics (at 1400 cm^{-1}) were noticeably absent.

The presence of the amide I and II bands in the sludge HA infrared spectrum (Fig. 6.1) indicated proteinaceous materials to be the major source of N enrichment of the sludge HA. That is, the increased N content of the sludge HA resulted from the association of proteinaceous materials with the sludge HA polymers. The presence of proteinaceous materials in sewage sludge has been demonstrated. Amino acid N has been shown to be the predominant form of organic N present in several sewage sludges (Sommers et al., 1972; Ryan et al., 1973). Organic fractions separated from sewage sludge using a heavy liquid technique were shown to contain significant quantities of proteinaceous materials as indicated by the predominance of the amide I and II bands in their ir spectra (Section 5). Characterization of the amino acids in sludge has shown that most common amino acids are found in substantial concentrations and that several amino acids of predominantly microbial origin (e.g., ornithine, α-amino-butyric acid) are also present.

The sludge HA could be formed either during anaerobic digestion or subsequently, while the sludge was being air-dried. The similarity of the ir spectrum of the sludge HA (Fig. 6.1) to those of recent lake sediment HA's (Otsuki and Hanya, 1967) suggested that the sludge HA developed during anaerobic digestion. The low amounts of carboxyls and phenolic hydroxyls in the sludge HA may be a reflection of the anaerobic conditons prevailing during synthesis.

The sludge HA infrared spectrum had absorption bands corresponding to C-H stretching modes of aliphatic methyl and methylene groups (near 2900 cm^{-1}) that were stronger and more differetntiated as compared to the soil HA shown here and others reported in the literature (Stevenson and Goh, 1971). It is likely that these bands originated from the long alkyl chains of fatty acids which are known components of sludge (Section 3). Schnitzer and Neyroud (1975) sampled two humic materials from widely different environments and established that straight chain fatty acids accounted for nearly 10% of the HA weight. Of the total fatty acids present, 95% were shown to be 'tightly held' probably through esterification to phenolic hydroxyls, and 5% were 'loosely held'. The loosely held fatty acids were most effectively extracted by sonification of an aqueous HA suspension followed by extraction with hexane. This procedure was not especially effective in removing alkanes, but did remove ~ 5% of the total fatty acids. Using the sludge HA, the sonification/hexane extraction procedure was performed and the amount of material removed was estimated by determining the % reduction in absorbance of the methylene C-H band at 2928 cm^{-1} (Fig. 6.4). The amide I bands, which should be unaffected by the extraction procedure, were mathematically scaled in absorbance mode such that they were equal in intensity, and the peak heights at 2928 cm^{-1} were measured from the

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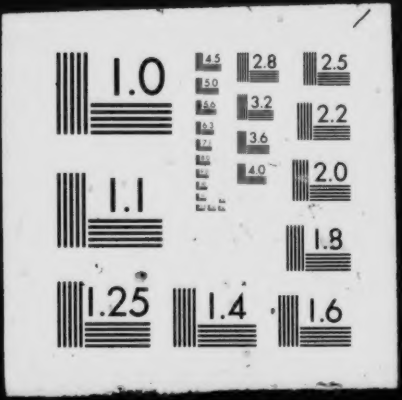

1.0
4.5
5.0
5.6
6.3
7.1
8.0
2.8
3.2
3.6
4.0
2.5
2.2
2.0
1.1
1.8
1.25
1.4
1.6

baselines. As a result of hexane extraction, the absorbance at 2928 cm^{-1} was reduced by 15.7%. This indicated that ~ 16% of the materials contributing to the 2928 cm^{-1} absorbance could be termed as being 'loosely held'. Assuming these materials were comprised primarily of fatty acids, and possibly smaller amounts of alkanes, this represented a significant increase over the 5% 'loosely held' fatty acids extracted from soil HA's (Schnitzer and Neyroud, 1975).

E_4/E_6 values (Table 6.2) were obtained for each [illegible] the HA's for use as a possible indicator of degree of humification. In this study the E_4/E_6 ratio appeared to be a relatively insensitive parameter for evaluating degree of humification. The Chalmers soil HA and HA's extracted from the sludge-amended soil, all gave essentially the same E_4/E_6 value of 5. The Chicago sludge HA gave the lowest E_4/E_6 value (3.8) of the HA's measured. It has been suggested that progressive humification and increased condensation, which infer the presence of relatively small proportions of aliphatic structures, are indicated by a decrease in the E_4/E_6 ratio (Kononova, 1966). Of the HA's studied, the Chicago HA, which certainly had the most aliphatic structure (as described above), gave the lowest E_4/E_6 value in direct opposition to the suggested relationship between E_4/E_6 and degree of humification. In fact, Chen et al. (1977), found that the E_4/E_6 ratio was not directly related to degree of humification. It was shown that the E_4/E_6 ratio of HA was governed primarily by particle size or molecular weight (negative correlation) of the material. Other secondary correlations existed between the E_4/E_6 ratio and % C (negative), total acidity (positive) and CO_2H groups (positive). The low E_4/E_6 ratio of the sludge HA, as compared to the Chalmers HA, may have been related to one or more of these analytical measurements but apparently not to degree of humification.

Humic acids extracted from the sludge-amended soils had increased N contents, lower C:H ratios, lower values of total acidity and phenolic OH, somewhat lower values of CO_2H groups, and lower absorptivities (Tables 6.1 and 6.2) as compared to the Chalmers HA. The effect of sludge amendment on these properties was greatest for HA extracted from the sludge-amended soil 2 months after application and was somewhat less apparent in HA extracted 14 months after application.

Infrared spectral data supported the results of elemental and functional group analyses. The ir spectrum of HA extracted from the sludge-amended soil 2 months after application reflected the increased N content, and decreased C:H ratio and phenolic OH content as compared to the Chalmers soil HA. That is, in the ir spectrum (Fig. 6.1) of HA extracted from the sludge-amended soil after 2 months, the increased N content was reflected by a marked increase in the intensity of the amide I band and an increase in the amide II band. The decreased C:H ratio was reflected by an increase in the intensity of the methylene C-H stretching vibration (2928 cm^{-1}). The decreased phenolic OH content was reflected by a decrease in the intensity of the band at 1400 cm^{-1}, which corresponded to O-H deformation and C-O stretch of phenolic OH.

The infrared spectrum of HA extracted from the sludge-amended soil 14 months after application showed the same trends but to a lesser extent (Fig. 6.1). For HA extracted 14 months after sludge application the intensities of the amide I and II bands and methylene C-H stretching vibrations were still increased as compared to the Chalmers HA but this increase was less than those observed in HA extracted 2 months after sludge application. These intermediate intensities of the amide I and II bands and the methylene C-H vibrations corresponded to the values of % N and C:H (for HA extracted 14 months after application) which were also intermediate between those of the Chalmers HA and HA extracted 2 months after sludge application (Table 6.1). Likewise, the intensity of the 1400 cm^{-1} band (O-H deformation and C-O stretch of phenolic OH) was intermediate for HA extracted 14 months after application, corresponding to its intermediate value of phenolic OH (Table 6.2).

The data presented indicated that sludge organic matter was recovered in HA fractions of the sludge-amended soil 2 and 14 months after sludge application. Proteinaceous and aliphatic (e.g., fatty acids) materials were the primary sludge constituents associated with HA's extracted from sludge and sludge-amended soil. These constituents were indicated by the increased intensity of the amide and C-H infrared absorptions, the increased N contents and the decreased C:H ratios and absorptivities of HA extracted from sludge and sludge-amended soil as compared to HA from soil not receiving sludge amendment.

The association of sludge borne proteinaceous and aliphatic materials with the HA fraction of the sludge amended soil could be a reflection of simple mixing of sludge and soil HA after incorporation of sludge into soil. The small amount of sludge HA mixed with native soil HA suggested that mixing was of minor importance. If the addition of sludge to soil significantly increased the degradation of native soil organic matter, as has been suggested in studies with ^{14}C labeled synthetic sludge (Terry et al., 1979a), the effect of mixing would be enhanced. Even though the data presented were consistent with the above study (Terry et al., 1979a) in which it was suggested that sludge addition increased the decomposition of soil organic matter, research with plant residues has not conclusively demonstrated the importance of a 'priming effect' (e.g., Jenkinson, 1971).

The observed association of sludge borne organic materials with HA fractions of the sludge-amended soil could also arise from the decomposition and/or alteration of sludge organic matter after its incorporation into soil such that increased amounts of sludge constituents were extractable into the HA fraction. It should be noted that the recovery of ^{14}C labeled synthetic sludge as HA was increased significantly during a 168 day lab incubation in Chalmers soil (Terry et al., 1979a).

The observation of proteinaceous materials associated with sludge HA and HA's extracted from sludge-amended soil as long as 14 months after application suggested that these proteinaceous materials were resistant to decomposition, possibly as a result of their incorporation into the HA polymer. The incorporation of proteinaceous materials into the HA structure has been demonstrated using model HA's. Haider et al. (1965) found that, in the presence of phenol oxidase, peptides were linked to the phenolic polymers formed from a mixture of hydroxy toluenes and benzoic acids. Several soil fungi have been shown to produce phenolic polymers which contain polypeptides and resemble soil HA's (e.g., Martin and Haider, 1969).

In the present study, processes similar to these may be responsible for the incorporation of sludge- borne proteinaceous materials into the sludge and sludge-amended soil HA polymers. The apparent resistance of these sludge-borne proteinaceous materials is consistent with previous studies whereby the incorporation of proteins, peptides, and amino acids (Verma et al., 1975) and proteinaceous materials of microbial origin (Verma and Martin, 1976; Nelson et al., 1979) into model HA-like phenolic polymers resulted in decreased decomposition of residual protein materials in soils.

The nature of the association of aliphatic materials, probably fatty acids, with HA's extracted from sludge-amended soil may be in part through esterification to phenolic hydroxyls as suggested by Schnitzer et al. (1975) for other HA's. The decreased phenolic OH content of these HA's, as compared to HA acid extracted from soil not receiving sludge amendment, was consistent with the esterificatin mechanism.

SUMMARY

Humic acids extracted from sludge, soil, and sludge-amended soil were characterized by elemntal and functional group analyses and visible and infrared spectroscopy. Humic acid (HA) extracted from a Chalmers soil appeared to be similar to an average HA (Schnitzer, 1978). Humic acid extracted from Chicago sludge, as compared to the average HA and Chalmers HA, had a lower C:H ratio, increased N content, decreased total acidity and carboxyl content, and a lower absorptivity. With respect to the average HA and Chalmers HA, the sludge HA appeared to be a more aliphatic, N-enriched polymer with decreased amounts of carboxyls and phenolic hydroxyls. Proteinaceous materials associated with the sludge HA appeared to be the major source of N enrichment. In general, HA's extracted from the sludge-amended soil had increased N contents, lower C:H ratios, lower values of total acidity and phenolic OH, somewhat lower values of CO_2H groups and lower absorptivities as compared to HA from soil not receiving sludge amendment. Sludge organic matter was apparently recovered in HA extracted from soil 2 and 14 months after sludge application. The association of sludge-borne proteinaceous and aliphatic materials with the sludge and sludge-amended soil HA fractions was indicated by increased amide and C-H stretch ir absorptions, as well as the increased N contents and decreased C:H ratios. The observation of proteinaceous materials associated with HA extracted from sludge-amended soil as long as 14 months after application suggested these proteinaceous materials were resistant to decomposition, possibly as a result of their incorporation into the HA structure. Fatty acids may be associated with HA's extracted from sludge and sludge-amended soil through esterification to phenolic hydroxyls.

Table 6.1. Elemental analysis and yield of humic acids.

Humic acid	Yield	Ash	C	H	N
	%[*]	----------------	%[+]	----------------	
Chalmers soil					
Non-amended	1.1	5.0	49.16	4.86	3.94
Sludge-amended (14 mo.)[§]	1.2	5.3	49.05	5.02	4.54
Sludge-amended (2 mo.)[§]	1.3	5.3	50.34	5.30	5.02
Chicago sludge	1.3	2.0	52.00	7.21	8.78

[*] % on ash free, moisture free basis

[+] % on moisture free basis

[§] After sludge application

Table 6.2. Functional group content, E_4/E_6 and absorptivity of humic acids.

Humic acid	Total acidity	CO_2H	Phenolic[*] OH	E_4/E_6	a[+]
	----- meq/g	humic acid[§]	-----		g^{-1} cm^{-1}
Chalmers soil					
Non-amended	7.2	2.6	4.6	5.0	4.7
Sludge-amended (14 mo.)[++]	5.1	2.5	2.6	5.2	4.1
Sludge-amended (2 mo.)[++]	4.0	2.2	1.8	5.1	3.4
Chicago sludge	2.4	0.95	1.45	3.8	1.1

[*] Phenolic OH = total acidity - CO_2H acidity

[+] Absorptivity at 465 nm

[§] On an ash free, moisture free basis

[++] After sludge application

Table 6.3. Infrared band assignments for the Chicago sludge humic acid.

Frequency, cm^{-1}	Assignment	Intensity*
3370 (broad)	Polymeric OH stretch H-bonded	vs
3200	N-H stretch of secondary amide (bonded)	sh
3075	N-H mode of secondary amide, aromatic C-H stretch	w
2957, 2875	Methyl C-H stretch	m
2928, 2855	Methylene C-H stretch	s
2700-2400	O-H stretch of carboxylic acid (bonded)	w
1720	C=O stretch of carboxylic acids and esters	sh
1655	C=O stretch of secondary amide (amide I)	vs
1520	Amide II band	s
1455	C-H deformation of $C-CH_3$ (asymmetrical and $-CH_2-$, aromatic skeletal ring breathing mode)	m
1385	C-H deformation of $C-CH_3$ (symmetrical)	w
1240	C-O stretch or O-H deformation of carboxylic acid, amide II band, aryl ethers (?)	m
1040	C-O stretch of polysaccharide	m

* vs, very strong; s, strong; m, medium; w, weak; sh, shoulder

Table 6.4. Infrared band assignments for humic acids extracted from Chalmers soil with and without sludge amendment.

Frequency, cm^{-1}	Assignment	Intensity*		
		Chalmers	2 mo.+	14 mo.+
3390	Polymeric O-H stretch H-bonded	s	s	vs
3075	Aromatic C-H stretch, N-H mode of secondary amide	w	w	w
2960	Methyl C-H stretch	-	w	w
2928, 2855	Methylene C-H stretch	w	m	m
1718	C=O stretch of carboxylic acid	sh	sh	sh
1655	C=O stretch of secondary amide (amide I)	m	s	vs
1635	C=O stretch of carboxylic acid H-bonded	s	m	m
1535	Amide II	w	m	m
1455	C-H deformation of $C-CH_3$ (asymmetrical) and $-CH_2-$	w	w	w
1400	O-H deformation and C-O stretch of phenolic OH	w	w	w
1230	C-O stretch or O-H deformation of carboxylic acid, amide II band, aryl ethers (?)	m	m	m
1040	C-O stretch of polysaccharide	m	m	m

* vs, very strong; s, strong; m, medium; w, weak; sh, shoulder

+ Months after sludge amendment

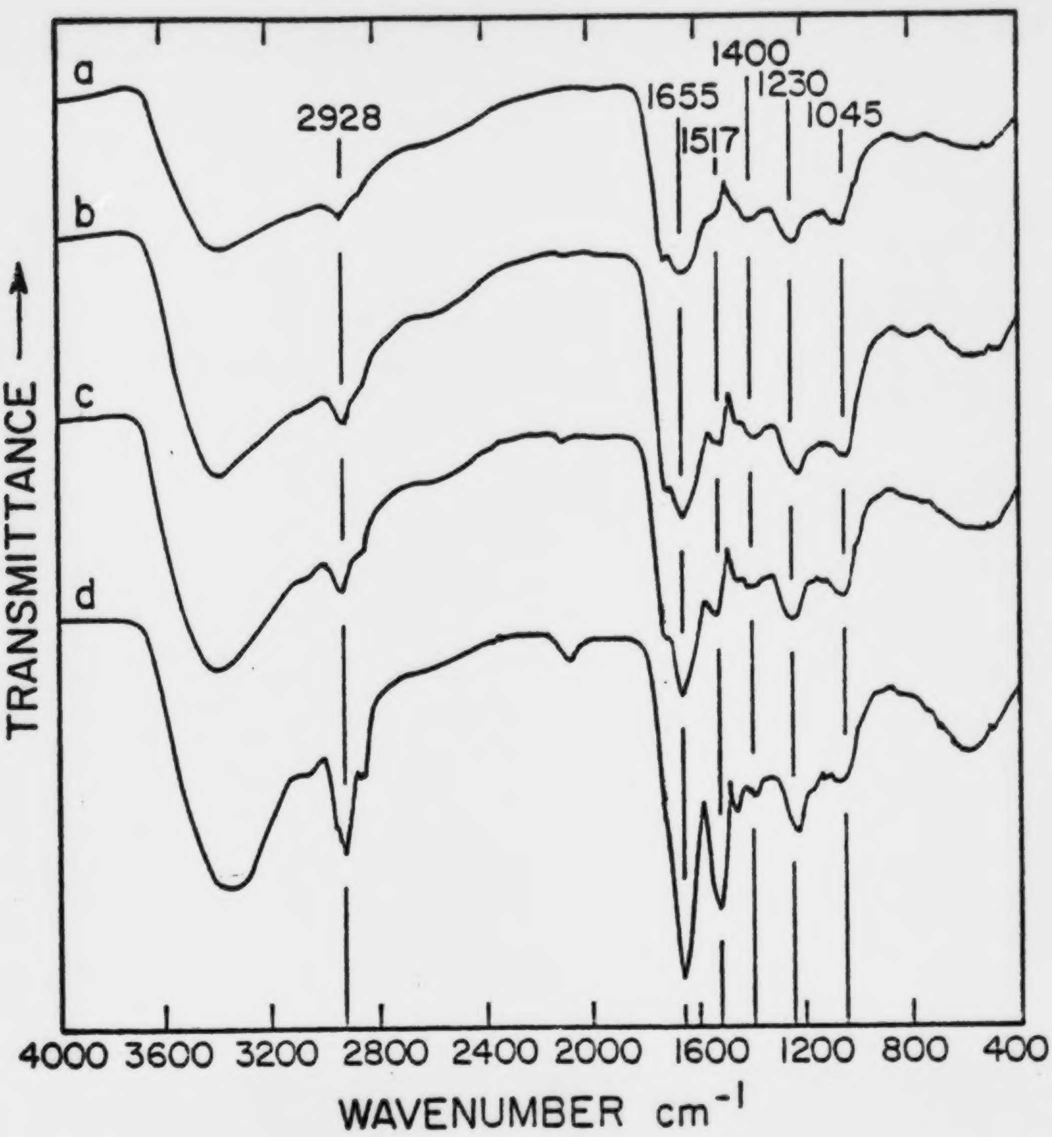


Figure 6.1. Infrared spectra of humic acids extracted from the (a) Chalmers soil, (b) sludge-amended soil 14 months after application, (c) sludge-amended soil 2 months after application, and (d) Chicago sludge.

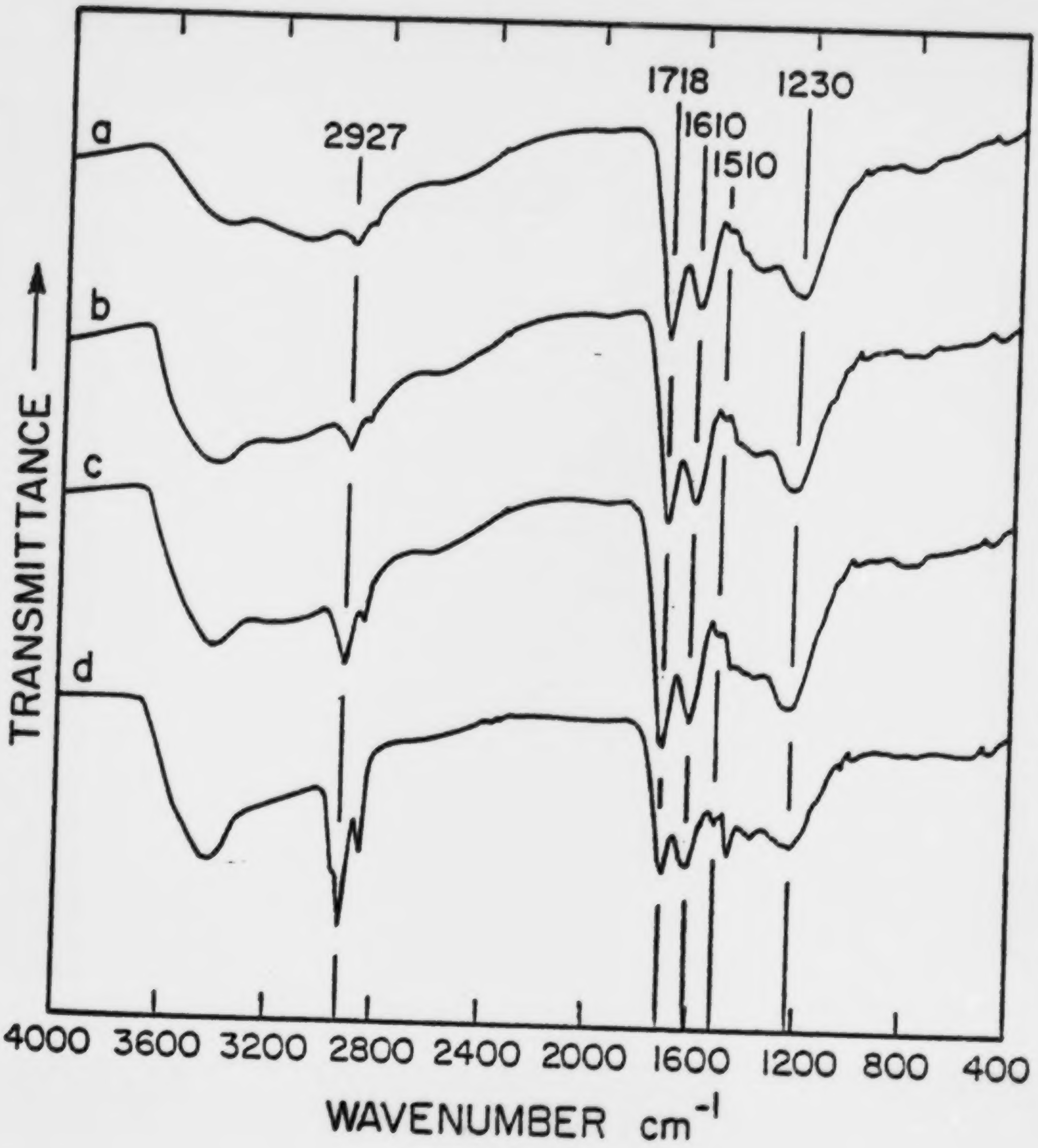


Figure 6.2. Infrared spectra of 6 $\underline{N}$ HCl hydrolyzed humic acids extracted from the (a) Chalmers soil, (b) sludge-amended soil 14 months after application, (c) sludge-amended soil 2 months after application, and (d) Chicago sludge.

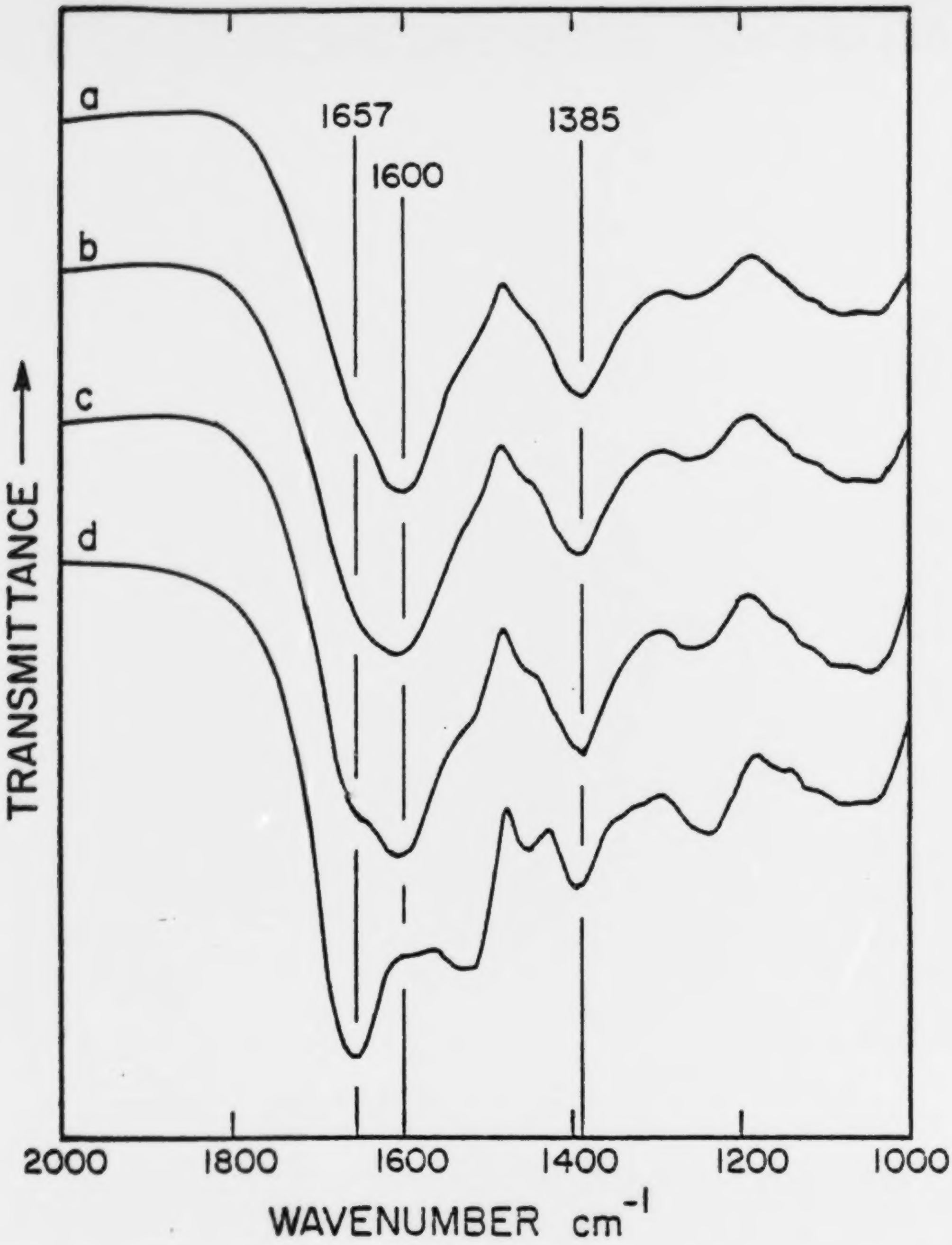


Figure 6.3. Infrared spectra of Na salts of humic acids extracted from the (a) Chambers soil, (b) sludge-amended soil 14 months after application, (c) sludge-amended soil 2 months after application and (d) Chicago sludge.

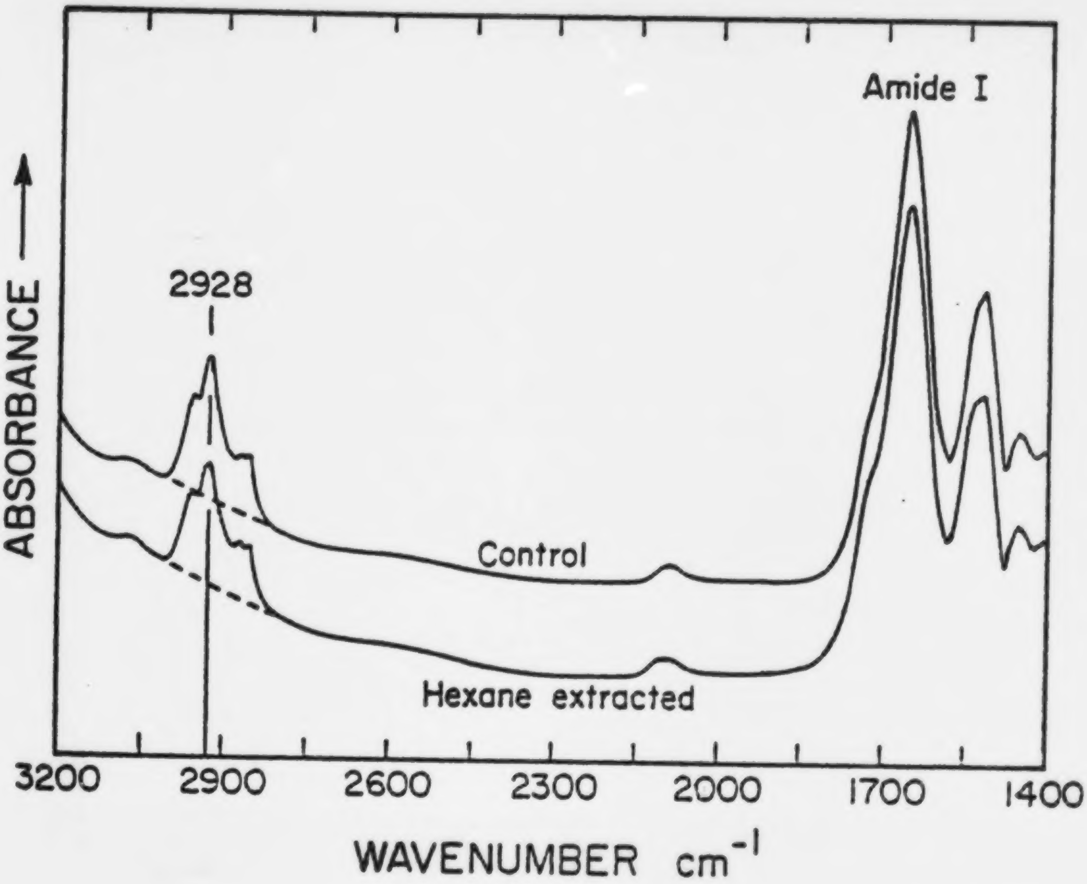


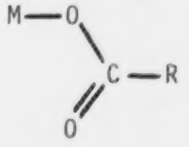
Figure 6.4. The effect of hexane extraction on the infrared spectra of humic acid recovered from the Chicago sludge.

SECTION 7

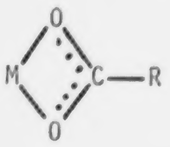
COPPER (II) AND IRON (III) COMPLEXATION BY THE CARBOXYLATE GROUP OF HUMIC ACID

INTRODUCTION

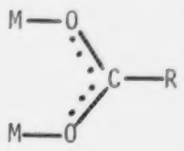
Many workers have implicated carboxylic acid (COOH) groups in the reaction of Cu^{2+} and Fe^{3+} with humic acid (Gamble *et al.*, 1970; Schnitzer, 1978; Schnitzer and Kahn, 1972; Schnitzer and Skinner, 1965; Stevenson and Ardakani, 1972; Van Dijk, 1971). It is generally considered that the reaction of metal ions such as Cu^{2+} and Fe^{3+} with humic acid (HA) results in the formation of a chelate ring involving adjacent COOH and phenolic OH groups or, less predominantly, two adjacent COOH groups. Alternatively, metal ions may be complexed by the same types of functional groups but not resulting in the formation of a chelate ring. Electron spin resonance (ESR) spectroscopy was used to show that Cu^{2+} formed two equatorial bonds with oxygen donor ligands of HA extracted from soil and sewage sludge (Section 8). The ESR data presented indicated the formation of a Cu-chelate in the Cu-HA complexes. Carboxylic acid groups were assumed to be involved in the binding of Cu^{2+} by these HA's. However, the carboxylate ion may complex ions in any of the following ways (Nakamoto, 1978):



I



II



III

When the COOH group is converted to CO_2^-, the ir absorption for carboxyl C=O is replaced by two bands near 1600 and 1400 cm^{-1} corresponding to the antisymmetrical and symmetrical stretching vibrations of CO_2^- (Bellamy, 1975). For a metal carboxylate complex, the separation between the antisymmetrical, $\nu_s(CO_2^-)$, stretching frequencies is related to the mode of complexation. The separation of $\nu_a(CO_2^-)$ and $\nu_s(CO_2^-)$, referred to below as Δ, is larger in unidentate complexes (structure I) than in the free carboxylate ion. In bidentate (chelate) complexes (structure II) Δ is smaller than in the free carboxylate ion, and in bridging complexes (structure III), is close to the value for the free carboxylate ion. The relationship between Δ and the mode of complexation by CO_2^- can be illustrated by referring to a study of the benzoates of Pr, Nd, Er and Tm

(Taylor et al., 1968). These rare earth metals were shown to form bidentate complexes (structure II) with benzoate. The bands, $\nu_a(CO_2^-)$ and $\nu_s(CO_2^-)$, reported for this series of rare earth benzoates occurred at ~ 1515 and 1400 cm^{-1}, respectively, giving Δ values of ~ 115 cm^{-1}. As predicted, these values of Δ were smaller than the corresponding value of Δ for the free benzoate ion (169 cm^{-1}, Table 7.1) due to the formation of the bidentate structure II.

In this study, Cu^{2+} and Fe^{3+} complexes of a soil HA were prepared and spectra were recorded between 2000 and 1000 cm^{-1}. The value of Δ for each complex was obtained from the data and compared to that in the free ion. The data confirmed that COOH groups of HA formed coordinate bonds with Cu^{2+} and Fe^{3+}. The objective of this study was to identify the mode (structure I, II, or III) by which carboxylates of HA complexed Cu^{2+} and Fe^{3+}.

MATERIALS AND METHODS

Humic acid (HA) was extracted from a Chalmers soil, purified and the Cu^{2+}-HA complexes prepared as described in previous sections. The Fe^{3+}-HA complex was prepared in the same fashion using $Fe(ClO_4)_3\ 6H_2O$. Infrared spectra were recorded with a Perkin-Elmer Model 180 double beam grating spectrophotometer. Samples (KBr discs) were prepared and ir spectra recorded as described previously.

RESULTS AND DISCUSSION

Infrared spectra of a Chalmers soil HA and the Cu^{2+}-HA and Fe^{3+}-HA complexes are shown in Fig. 7.1. Table 7.1 gives experimentally determined ir frequencies, $\nu_a(CO_2^-)$ and $\nu_s(CO_2^-)$, for the Cu^{2+}-HA and Fe^{3+}-HA complexes along with values from the literature for benzoate and salicylate anions (Dunn and Mcdonald, 1969). The values of Δ [$\Delta = \nu_a(CO_2^-) - \nu_s(CO_2^-)$] listed in Table 7.1 for the benzoate and salicylate ions were used as reference values for the free carboxylate ion since the COOH groups of these acids are representative of those found in HA (Schnitzer, 1978). The IR frequencies of the benzoate and salicylate ions were obtained from the sodium salts in D_2O solution (Dunn and McDonald, 1969).

Infrared band assignments for the Chalmers soil HA have been reported. In the 2000-1000 cm^{-1} region of the soil HA spectrum (shown in Fig. 7.1) the 1718 cm^{-1} band was assigned as the carboxyl C=O stretching vibration and the 1655 to 1620 cm^{-1} absorption was assigned as H-bonded carboxyl stretching modes. In the Cu^{2+} and Fe^{3+} complexes the carboxyl stretching vibrations were replaced by two bands at ~ 1620 and 1385 cm^{-1} corresponding to the antisymmetrical, $\nu_a(CO_2^-)$, and symmetrical, $\nu_s(CO_2^-)$, vibrations of the carboxylate ion. Thus, the carboxyl absorptions at 1720 cm^{-1} and 1655 to 1620 cm^{-1} in the IR spectrum of the soil HA originated largely from COOH groups and these COOH groups were converted to CO_2^- by forming coordinate bonds with Cu^{2+} and Fe^{3+}.

The values of Δ for the Cu^{2+}-HA and Fe^{3+}-HA complexes were larger than the corresponding free carboxylate ion reference values of Δ for the benzoate and salicylate ions (Table 7.1). Thus, COOH groups of HA formed unidentate complexes (structure I) with Cu^{2+} and Fe^{3+}.

Data in Section 8 shows that Cu^{2+} formed two equatorial bonds with oxygen donor ligands of HA. The ESR data obtained indicated the formation of a chelate ring in the original Cu-HA complex. The results presented above show that these two equatorial bonds do not result from the formation of a bidentate Cu^{2+} complex with a single COOH group (structure II). However, the above results were consistent with the formation of a chelate ring involving adjacent COOH groups each forming single bonds (structure I) with Cu^{2+}. Similarly, the formation of a chelate ring in the Cu-HA complex could result from the complexation of Cu^{2+} by a COOH group, as illustrated in structure I, and an adjacent phenolic OH.

SUMMARY

Infrared spectroscopy was used to identify the mode by which carboxylates of a soil humic acid (HA) complexed Cu^{2+} and Fe^{3+}. Infrared spectral data of soil HA and the Cu^{2+}-HA and Fe^{3+}-HA complexes were recorded between 2000 and 1000 cm^{-1}. Carboxylic acid groups of HA were converted to carboxylates (CO_2^-) by forming coordinate bonds with Cu^{2+} and Fe^{3+}. The separation (Δ) of the antisymmetrical, $\nu_a(CO_2^-)$, and symmetrical, $\nu_s(CO_2^-)$, stretching vibrations of CO_2^-, was obtained from the IR spectra of the metal-HA complexes. The values of Δ for the metal-HA complexes were significantly larger than the corresponding reference value of Δ for free CO_2^-, demonstrating that CO_2^- formed a unidentate complex (i.e., a single M-O bod) with Cu^{2+} and Fe^{3+}. The formation of bidentate or bridging complexes between CO_2^- of HA and Cu^{2+} or Fe^{3+} was not observed.

Table 7.1. Carboxylate stretching frequencies.

Compound	$\nu_a(CO_2^-)$	$\nu_s(CO_2^-)$	Δ	Structure
	------- cm^{-1}	-------		
Benzoate*	1548	1391	157	free ion
Salicylate*	1546	1377	169	free ion
Cu^{2+}-HA	1614	1385	229	unidentate
Fe^{3+}-HA	1625	1384	241	unidentate

* From Dunn and McDonald (1969)

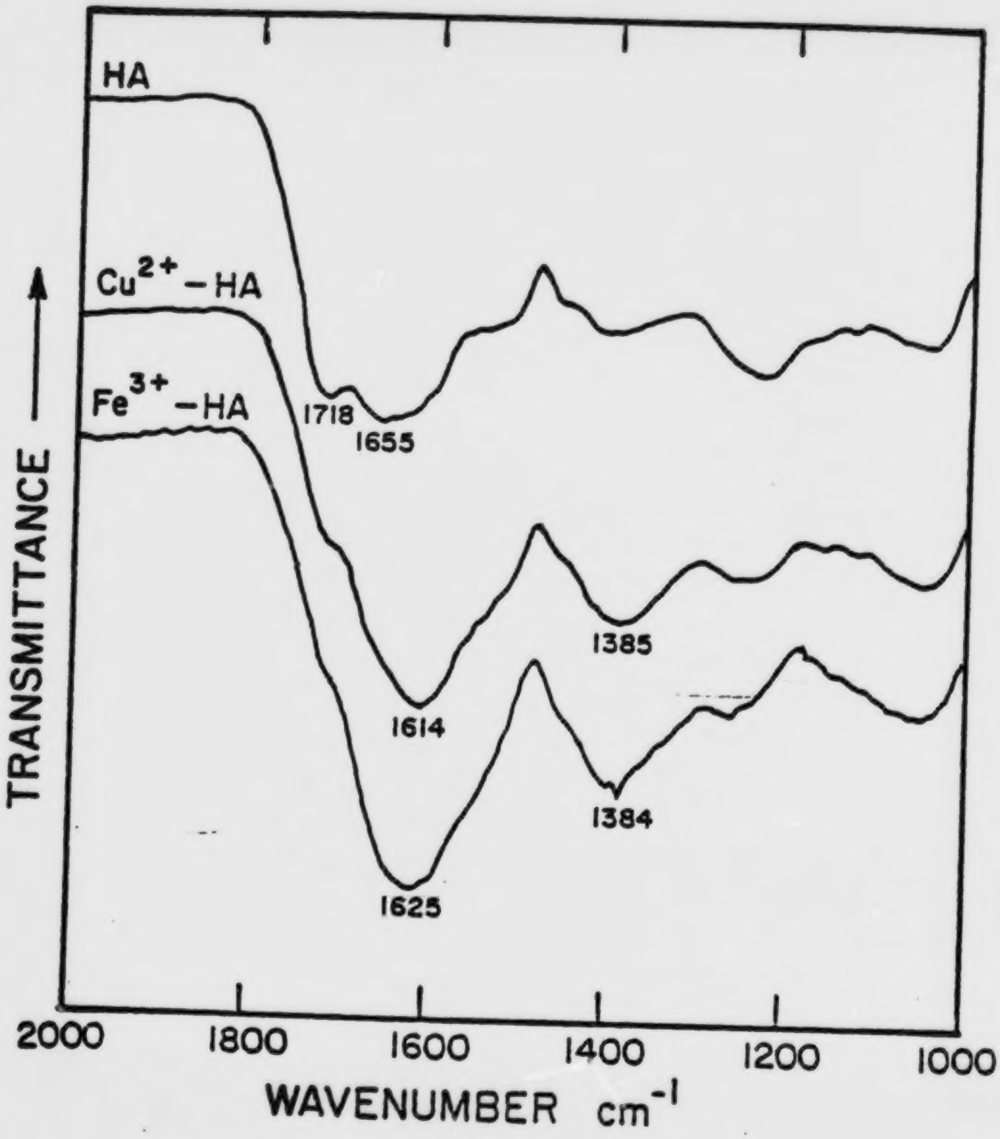


Figure 7.1. Infrared spectra of the Chalmers soil humic acid (HA) and the Cu^{2+}-HA and Fe^{3+}-HA complexes.

SECTION 8

THE MECHANISM OF COPPER (II) BINDING BY HUMIC ACID EXTRACTED FROM SOIL AND SEWAGE SLUDGE: AN ELECTRON SPIN RESONANCE STUDY

INTRODUCTION

One of the most important properties of humic and fulvic acids is their ability to interact with metal ions. There has been considerable interest in the reaction of soil organic matter with metals as evidenced by several review articles (Flaig et al., 1975; Jellinek, 1974; Mortensen et al., 1963; Schnitzer and Kahn, 1972; Stevenson and Ardakani, 1972). Knowledge of metal binding sites in soil organic matter is important from the standpoint of the chemistry of micro elements and may contribute to the development of a more valid description of the structure of humic materials in soils.

The cupric ion is complexed strongly by humic and fulvic acid (Schnitzer and Kahn, 1972, and references therein). The reaction of Cu^{2+} with humic and fulvic acid has generally been considered to involve chelation by o-hydroxycarboxylic acid (e.g., salicylic acid) and o-dicarboxylic acid (e.g., phthalic acid) moieties, the former being predominant (Gamble et al., 1970; Schnitzer and Kahn, 1972; Schnitzer and Skinner, 1965; Stevenson and Ardakani, 1972; Van Dijk, 1971). Alternatively, metal ions may be complexed by the same types of oxygen containing functional groups but not resulting in the formation of a chelate ring.

Recently, electron spin resonance (ESR) spectroscopy has been used to study transition metal binding to soil organic matter (Bloom and McBride, 1979; Bresnahan et al., 1978; Goodman and Cheshire, 1973; Lakatos et al., 1977; McBride, 1978). Copper bound to peat humic acid (HA) after repeated acid washings was studied with ESR (Goodman and Cheshire, 1973). It was concluded that this strongly bound unavailable Cu^{2+} was coordinated to porphyrin derivatives in peat HA. Lakatos et al. (1977) recorded the ESR spectrum of a Cu^{2+}-peat HA complex which had been dried at 130°C, and reported the Cu^{2+} was bound by two ligands containing aliphatic nitrogen donors and two carboxylates.

The binding of Cu^{2+} to HA extracted from a mineral soil was studied using ESR (McBride, 1978). Based on the observation of an anisotropic Cu^{2+} ESR spectrum, it was concluded that Cu^{2+} formed an inner sphere complex with HA ligands containing oxygen donors. Both Cu^{2+} and VO^{2+} formed such inner sphere complexes with HA whereas Mn^{2+} formed an outer sphere complex. Due to the Jahn Teller distortion (Cotton and Wilkinson, 1972), Cu^{2+} and VO^{2+} bind axial ligands weakly (those along the z axis, Fig. 8.1) and McBride

(1978) suggested that this may enable HA functional groups to displace axially coordinated H_2O ligands resulting in the formation of an inner sphere complex. By comparison, Mn^{2+} does not undergo the Jahn Teller distortion and so the $Mn(H_2O)_6^{2+}$ ion has no labile H_2O ligands. This reasoning was used to explain the formation of an outer sphere complex between Mn^{2+} and HA. In addition to axial coordination of Cu^{2+} by oxygen containing functional groups of HA, McBride (1978) stated that there was no evidence for the formation of more than a single bond between HA and Cu^{2+}.

Bloom and McBride (1979) used ESR spectroscopy to study Cu^{2+} binding to peat. The Cu^{2+} ions on peat exchange sites gave anisotropic spectra suggesting that at least one H_2O ligand of $Cu(H_2O)_6^{2+}$ had been displaced by the carboxylate oxygen of peat, resulting in the formation of an inner sphere complex. The ESR parameters obtained for the Cu^{2+}-peat complex indicated that Cu^{2+} was bound by ligands containing oxygen donors and there was no evidence of coordination by ligands containing nitrogen donors. They described the binding of Cu^{2+} by peat as involving the exchange of one or two axial aquo ligands of $Cu(H_2O)_6^{2+}$ by carboxylate oxygens because of the Jahn Teller distortion. It was concluded that chelation of Cu^{2+} by adjacent functional groups (e.g., o-hydroxybenzoic acid) was not significant in soil organic matter.

Thus, McBride (1978) and Bloom and McBride (1979) suggested a mechanism for Cu^{2+} binding to HA and peat whereby ligands containing oxygen donors (e.g., carboxylates) displaced weakly bound axial aquo ligands 'coordinated' to $Cu(H_2O)_6^{2+}$ resulting in the formation of one or two axial bonds to Cu^{2+}. The proposed mechanism is quite different from the general description of Cu^{2+} binding by HA because it has generally been thought that at least two equatorial Cu-HA oxygen bonds are formed (those in the x-y or equatorial plane, Fig. 8.1), probably via chelation (e.g., Schnitzer and Kahn, 1972; Stevenson and Ardakani, 1972; Van Dijk, 1971).

A related study compared the HA extracted from sewage sludge, Chalmers soil, and Chalmers soil treated with sewage sludge, through the use of infrared and visible spectroscopy, and elemental and functional group analyses (Boyd et al., 1980a). The sewage sludge HA was characterized as a more aliphatic, nitrogen enriched polymer with lower amounts of carboxylic acid groups and phenolic hydroxyls, as compared to an average soil HA (Schnitzer, 1978) and the Chalmers soil HA. Proteinaceous materials associated with the sludge HA were the major source of nitrogen enrichment. The E_4/E_6 ratio of sludge HA was lower than that of the Chalmers soil HA. The lower E_4/E_6 ratio obtained for the sludge HA suggested an increased particle size as compared to the Chalmers soil HA (Chen et al., 1977). In view of these differences in the HA fraction of soil and sewage sludge, it was of interest to examine the bonding of Cu^{2+} to HA extracted from sewage sludge using the ESR method.

MATERIALS AND METHODS

Materials

Surface samples (0-15 cm) of a Chalmers silty clay loam (Typic

Argiaquoll, fine, mixed, mesic) were obtained from the Purdue University Agronomy Farm, W. Lafayette, IN, air dried and ground to pass a 40 mesh screen prior to extraction. The sewage sludge was obtained from Chicago, IL. Humic acid (HA) was extracted from the Chalmers soil and purified as described previously (Section 6). Elemental composition, functional group content, yield and ash of the Chalmers HA were presented in Section 6.

Cu-HA Complex

Following extraction and dialysis of the HA (Section 6) a concentrated HA suspension was recovered by centrifugation. Ten equal subsamples of the suspension were transferred onto separate Whatman No. 1 filter papers held on glass funnels. Each of the 10 HA subsamples was leached over a 6 hr period with 100 ml of a 0.1 M $Cu(ClO_4)_2\ 6H_2O$ solution (filtered through Whatma No. 42 filter paper prior to use) to form the Cu-HA complex. All 10 samples were subsequently transferred onto a single Whatman No. 1 filter paper held on a Buchner funnel and washed with 500 ml of deionized H_2O using vacuum filtration. The Cu-HA sample was then washed with 100 ml of a 0.001 N HCl solution and again washed with ~ 500 ml of deionized H_2O until a negative Cl^- test was obtained with $AgNO_3$. The washed Cu-HA complex was then mixed with ~ 50 ml deionized H_2O, shaken overnight and stirred several hours using a magnetic stirrer. From the resulting Cu-HA suspension, 1.5 ml aliquots were taken and placed in 1 dram glass vials and capped. The average weight of the Cu-HA complex contained in each vial (~ 0.05 g) was determined by weighing dried samples in triplicate on a Mettler B-6 Semi-Micro Balance (± 0.02 mg). The soil HA-Cu and sludge HA-Cu complexes contained 6.85% and 2.16% Cu, respectively.

Nitrogen Adducts for Soil HA

Aqueous solutions of pyridine (py) and ethylenediamine (en) were prepared at appropriate concentrations and 0.1 ml of each solution were added, using an Eppendorf pipet, to vials containing the Cu-HA complex. Similarly, 25% ethanol solutions of 1,10-phenanthroline (phen) were prepared and 0.2 ml of these solutions were added to vials containing the Cu-HA complex. In this fashion py was added at M:L (Cu:ligand) ratios of 1:1, 1:2, 1:4, 1:6, and 1:25, and en and phen were added at M:L ratios of 1:1 and 1:2. The 1:50 py adduct was prepared by adding py (neat) directly.

Nitrogen Adducts for Sludge HA

To prepare the adducts with py, phen, en, and glycylglycine (glygly), aqueous solutions of each ligand were prepared (25% ethanol solutions for phen) at various concentrations and 0.5 ml were added to each vial using an Eppendorf pipet. In this fashion, adducts were prepared with the following Cu:ligand (M:L) ratios: (i) 1:1, 1:2, 1:4, 1:6, 1:30 and 1:60 for py; (ii) 1:1 and 1:2 for phen and en; and (iii) 1:1 for glygly.

Electron spin resonance spectra were recorded on a Varian E104 spectrometer and calibrated with diphenylpicrylhydrazyl (dpph) as a g marker (g = 2.0036). Glassy spectra were recorded at 77°K in quartz tubes and room temperature spectra were recorded in a quartz flat face aqueous cell.

Abreviations used in this work are: HA = humic acid, py = pyridine, phen = 1,10-phenanthroline, en = ethylenediamine, glygly = glycylglycine, 5-ClSAL = 5-chlorosalicylate, 2,2-tri = 1,4,7-triazaheptane, bipy = bipyridyl.

RESULTS AND DISCUSSION

Soil Humic Acid-Copper (II) Complexes

Electron spin resonance (ESR) spectral parameters for the Chalmers soil humic acid (HA) treated with Cu^{2+} and washed free of excess Cu^{2+} with deinized H_2O are given in Table 8.1. The ESR spectrum of the Cu-HA complex recorded at 77°K was consistent with a $d_{x^2-y^2}$ (or d_{xy}) ground state and gave $g_\parallel$ and $A_\parallel$ values similar to those reported earlier for a soil Cu-HA complex (McBride, 1978). At room temperature the Cu-HA complex gave an anisotropic (non-rotating Cu^{2+}) spectrum (Table 8.1), indicating Cu^{2+} was immobilized by forming an inner sphere complex with HA. The formation of an inner sphere Cu^{2+} complex with soil HA was previously been demonstrated (McBride, 1978). The $g_\parallel$ and $A_\parallel$ values (Table 8.1) for the Cu-HA complex are typical of Cu^{2+} species coordinated with ligands having oxygen donor atoms. For example, Cu^{2+} complexes with equatorial coordination by two H_2O's and a dicarboxylic acid, e.g., $Cu(malonate)(H_2O)_2$, gave $g_\parallel$ and $A_\parallel$ values (Table 8.2) similar to those obtained for the Cu-HA complex. Several other CuO_4 chromophores reported in the literature are listed in Table 8.2. Therefore, we are convinced, based on these findings, that HA oxygen donors have displaced H_2O from the hydrated Cu^{2+} species, forming an inner sphere complex.

To a first approximation, g values for Cu^{2+} are given by:

$$g = 2.0023\left(1 + \frac{r\lambda}{\Delta}\right)$$

For Cu^{2+} in a tetragonal ligand field, r is -4 ($g_\parallel$) and -1 ($g_\perp$), λ is the spin orbit coupling constant and is -828 K (Kaiser) and Δ is the separation in energy of the d_{xy} and $d_{x^2-y^2}$ orbitals (Vanngard, 1972). Δ is obtained from optical absorption spectra and ranges from 14 to 18 kK. Ligands vary in their capacity to cause d orbital splitting and so Δ is variable. Ligands that give larger values of Δ are said to produce a stronger crystal field or a larger crystal field stabilization energy. The lower $g_\parallel$ values of the Cu-HA complex (Table 8.1) as compared to $Cu(H_2O)_6^{2+}$ (Table 8.2) demonstrated that the ligand field produced by oxygen donor ligands of HA was stronger (larger Δ) than that of H_2O, assuming these ligands were coordinated equatorially. The formation of axial Cu-O bonds involving HA oxygen donor ligands seems unlikely. Increasing the axial ligand field of tetragonal Cu^{2+} has been shown to result in increased $g_\parallel$ and decreased $A_\parallel$ values (Dejehet _et al._, 1978; Smith, 1970). The ligand field of the carboxylate group has been shown to be stronger than that of H_2O (Arena _et al._, 1977; Dawson _et al._, 1972). Displacement of axial H_2O ligands from $Cu(H_2O)_6^{2+}$ by carboxylate ligands of HA should result in an increase in $g_\parallel$ and a decrease in $A_\parallel$ relative to $Cu(H_2O)_6^{2+}$ (Table 8.2). However, the $g_\parallel$

and A‖ values reported here and others reported in the literature (Bloom and McBride, 1979; McBride, 1978) for Cu-HA complexes are lower and higher respectively than those of $Cu(H_2O)_6^{2+}$ (Table 8.2), in opposition to the expected behavior of these parameters if axial coordination by the functional groups of HA, such as carboxylate, had occurred. Thus, the oxygen donor ligands of HA appeared to be coordinated equatorially to the Cu^{2+} center.

It was of interest to examine further the nature of the Cu-HA complex in an attempt to reveal the mechanism of Cu^{2+} binding by HA. Information regarding the nature of the Cu-HA complex can be derived from its g‖ value. The g‖ value may yield information about the kind of atoms coordinating to the Cu^{2+} center and the strength of their interaction. In general, g‖ factors for Cu^{2+} decrease in the following order for different donor atoms: oxygen > nitrogen > sulfur (Vanngard, 1972). The g‖ value for Cu^{2+} decreases as the number of equatorially coordinated ligands containing nitrogen donors increases (Falk, 1970). The relationship between g‖ and equatorial nitrogen coordination is shown clearly by comparing g‖ values of the various copper chromophores listed in Table 8.2. Although less regular, a similar but opposite relationship exists between the number of equatorially coordinated nitrogen donors and A‖ . That is, A‖ values for Cu^{2+} increase as equatorial nitrogen coordination increases.

It has been shown experimentally and confirmed theoretically that the effects of axial nitrogen coordination on the ESR parameters are opposite the effects of equatorial nitrogen coordination (Falk, 1970). Thus, as the number of equatorial nitrogen ligands increases, g‖ decreases and A‖ increases, whereas, axial nitrogen coordination causes g‖ to increase and A‖ to decrease.

Several Cu-HA-N adducts were prepared by adding ligands containing nitrogen donors to the Cu-HA complex in an attempt to identify a nitrogen ligand that would selectively substitute the added ligand for coordinated H_2O without displacing HA oxygen donor ligands also coordinated to Cu^{2+} (Fig. 8.1). Identification of the resulting chromophore from its ESR spectral parameters would enable the determination of the number and location of bonds between Cu^{2+} and HA in the original Cu-HA complex. The Cu-HA-N adducts studied are listed in Table 8.1 with their corresponding g‖ and A‖ values. ESR spectal parameters obtained at room temperature are also given in Table 8.1.

Pyridine (py) was added to the Cu-HA complex at M:L ratios of 1:1, 1:2, 1:4, 1:6, 1:25 and 1:50 (Table 8.1). Pyridine has been shown to displace H_2O ligands coordianted to Cu^{2+} (Naccache and Taarit, 1971). Addition of py at M:L ratios of 1:1 and 1:2 caused g‖ to decrease and A‖ to increase resulting from equatorial nitrogen coordination. The g‖ and A‖ values of the 1:2 py adducts were similar to those reported in the literature for various CuO_2N_2 chromophores (Table 8.2). Both the 1:1 and 1:2 py adducts were non-rotating at room temperature, indicating that the HA ligands had not been displaced, i.e., the Cu^{2+} center remained immobilized. Thus, the addition of py at M:L ratio of 1:2 resulted in the displacement of two equatorial H_2O ligands and the formation of two equatorial Cu-N bonds, giving a CuO_2N_2 chromophore. When py was added at a M:L ratio of 1:4, the g$\|$ and A$\|$ values remained essentially the same as for the py adduct (Table 8.1) indicating that the addition of py at a M:L ratio of 1:4 caused no further substitution of py for H_2O ligands beyond that resulting from the addition of py at a M:L ratio of 1:2. In both the 1:2 and 1:4 py adducts, two equatorial H_2O ligands were substituted by py. The addition of py at a M:L ratio of 1:6 caused g$\|$ to increase slightly and A$\|$ to decrease as compared to the 1:2 adduct (Table 8.1). The g$\|$ value continued to increase and A$\|$ continued to decrease to their maximum (g$\|$ = 2.26) and minimum values (A$\|$ = 179), respectively, in the 1:25 and 1:50 py adducts (Table 8.1). The 1:6, 1:25 and 1:50 py adducts were all non-rotating at room temperature. The increased g$\|$ and decreased A$\|$ values in the 1:6, 1:25, and 1:50 py adducts, as compared to the 1:2 py adduct, indicated that the axial ligand field of the complex was strengthened resulting from axial coordination of py (Dejehet _et al_., 1978; Falk, 1970; Smith, 1970). Substitution of axially 'coordinated' H_2O ligands by py began in the 1:6 py adduct and these axial sites were fully occupied by py in the 1:25 and 1:50 py adducts, forming a CuO_2N_4 chromophore. [Note: CuO_2N_4 implies axial positions occupied by nitrogen donors. All other species have axial positions occupied by H_2O.]

To summarize the results of the py adducts, when py was added in a M:L ration of 1:2 and 1:4, two equatorially coordinated H_2O ligands were replaced with py. In the 1:6 py adduct, substitution of axial H_2O ligands by py began and was complete in the 1:25 and 1:50 adducts, which had two equatorial and two axial sites occupied by py with the remaining two equatorial sites of Cu^{2+} occupied by HA ligands containing oxygen donors. Room temperature ESR spectra of all the py adducts were anistropic (Table 8.1). Pyridine selectively displaced coordinated H_2O molecules from the Cu-HA complex without displacing HA oxygen donors also coordinated to the Cu^{2+} center, resulting in the formation of a CuO_2N_2 chromophore in the 1:2 and 1:4 py adducts and a CuO_2N_4 chromophore in the 1:25 and 1:50 py adducts. Based on this characterization of the py adducts, there were two HA oxygen donors coordinated equatorially to the Cu^{2+} center in the original Cu-HA complex, as illustrated in Fig. 8.1. An ESR study of VO^{2+} complexation in soil organics has shown that oxygen donor ligands of peat displaced an average of two H_2O ligands from $VO(H_2O)_5{}^{2+}$, forming an inner sphere complex (McBride, 1980).

The bidentate nitrogen donor 1,10-phenanthroline (phen) was also added to the Cu-HA complex. Addition of phen at M:L ratios of 1:1 and 1:2 resulted in adducts with g$\|$ and A$\|$ values similar to those of the 1:2 py adduct (Table 8.1) and to those reported in the literature for various CuO_2N_2 chromophores (Table 8.2). Both the 1:1 and 1:2 phen adducts were non-rotating at room temperature. These results indicated that the addition of phen at M:L ratios of 1:1 and 1:2 resulted in the substitution of two equatorially coordinated H_2O ligands by phen forming a CuO_2N_2 chromophore; a result analogous to and consistent with the 1:2 and 1:4 py adducts. 1,10-phenanthroline selectively displaced two equatorially coordinated H_2O ligands from the Cu^{2+} center, again indicating that there were two HA oxygen donor ligands coordinated equatorially in the original Cu-HA complex.

Another bidentate nitrogen donor, ethylenediamine (en), was added at M:L ratios of 1:1 and 1:2 (Table 8.1). As with the 1:1 phen and 1:2 py adducts, the 1:1 en adduct gave g$\|$ and A$\|$ values typical of a CuO_2N_2

chromophore. The 1:1 en adduct was determined to be non-rotating at room temperature, indicating that the HA oxygen donors had not been displaced. Referring to equation [1], the lower g∥ value of the 1:1 en adduct (Table 8.1), as compared to $[Cu(en)(H_2O)]^{2+}$ (Table 8.2), again demonstrated that the crystal field strength of HA oxygen donor ligands coordinated to Cu^{2+} was stronger (larger Δ) than that of H_2O. Addition of en at a M:L ratio of 1:2 gave g∥ and A∥ values typical of a CuN_4 chromophore, suggesting the formation of $[Cu(en)_2]^{2+}$. The observation of an isotropic spectrum (rotating Cu^{2+}) at room temperature for the 1:2 en adduct confirmed that $[Cu(en)_2]^{2+}$ had formed. It should be noted that the ESR parameters shown in Table 8.1 for this sample are in agreement with values reported by Barbucci and Campbell (1976) for $[Cu(en)_2]^{2+}$. Thus, en, which is a stronger base than py and phen, displaced not only coordinated H_2O but also the HA oxygen donors from Cu^{2+}.

The formation of a CuO_2N_2 chromophore resulting from the addition of the bidentate nitrogen donors phen and en at a M:L ratio of 1:1, and the observation that these adducts were non-rotating at room temperature, required the two equatorial Cu-HA oxygen bonds occupy cis positions in the square plane (x, y plane, Fig. 8.1), as shown in Fig. 8.1. If the two equatorial Cu-HA oxygen bonds occupied trans positions in the square plane, the formation of a CuO_2N_2 chromophore in the 1:1 phen and en adducts would be prohibited. Thus, the results obtained were consistent with the formation of a Cu^{2+}-chelate in the original Cu-HA complex. The formation of a chelate ring involving the bidentate nitrogen donors phen and en could possibly cause trans HA oxygen donor ligands to assume cis positions. However, such a rearrangement would be unfavorable sterically.

Sludge Humic Acid-Copper (II) Complexes

Electron spin resonance spectral parameters recorded at 77°K and room temperature for a Cu^{2+} complex of HA recovered from sewage sludge are given in Table 8.3. Also listed are ESR parameters of the adducts with py, phen, en, and glygly.

The sludge HA-Cu complex gave g∥ and A∥ values consistent with a CuO_4 chromophore in a $d_{x^2-y^2}$ (or d_{xy}) ground state. The room temperature spectrum was anisotropic (non-rotating Cu^{2+}) demonstrating that HA oxygen donor ligands had displaced H_2O from the hydrated Cu^{2+} species, forming an inner sphere complex. The formation of inner sphere Cu^{2+} complexes with HA oxygen donor ligands has been shown for soil HA's (McBride, 1978). The lower g∥ value of the sludge HA-Cu complex (Table 8.3) as compared to $Cu(H_2O)_6{}^{2+}$ (Table 8.2) indicated that the equatorial ligand field at the Cu^{2+} center had strengthened resulting from the formation of equatorial Cu-HA oxygen bonds. The g∥ value has been shown to decrease as the equatorial ligand field increases (Vanngard, 1972). The ligand fields produced by oxygen donor ligands of soil HA and the carboxylate group (Arena *et al*., 1977; Dawson *et al*., 1972) have been shown to be a stronger than that of H_2O. Similarly, the sludge HA ligands produced a stronger ligand field than H_2O.

The g∥ value of the sludge HA-Cu complex (g∥ = 2.36) was slightly higher (Table 8.3) than that observed for a soil HA-Cu complex (Table 8.1).

A strengthening of the axial ligand field of Cu^{2+} resulting from 'coordination' of sludge HA donor ligands at the axial sites could account for the increased g‖ value. Increasing the axial ligand field of Cu^{2+} has been shown to result in increased g‖ values (Dejehet et al., 1978; Smith, 1970). Thus, it appeared that sludge HA donor ligands were coordinated axially, as well as equatorially, to the Cu^{2+} center.

The sludge HA-Cu adducts with nitrogen donor ligands were prepared by adding py, phen, and en to the sludge HA-Cu complex, at various Cu:ligand (M:L) ratios, in an attempt to selectively substitute the added ligands for coordinated H_2O. The observation of an anisotropic spectrum at room temperature (non-rotating Cu^{2+}) showed that the added ligands had not displaced HA oxygen donor ligands coordinated to Cu^{2+}, i.e., the Cu^{2+} ion remained immobilized. The g‖ value of Cu^{2+} is known to be sensitive to the kinds of atoms coordianted (Vanngard, 1972) and can be used as a probe to observe the substitution of H_2O ligands by nitrogen donor ligands. As the number of equatorially coordinated nitrogen donor atoms increases, g‖ decreases (Falk, 1970). Although less regular, a similar but opposite trend is observed for A‖ which increases with the number of equatorially coordinated nitrogen donor ligands. The relationship between g‖ and equatorial nitrogen coordination is shown clearly in Table 8.2, which lists various Cu^{2+} chromophores reported in the literature and their corresponding g‖ and A‖ values. It has been shown experimentally (Falk, 1970; Dejehet et al., 1978) and confirmed theoretically (Falk, 1970; Smith, 1970) that the effects of axial nitrogen coordination on the Cu^{2+} ESR parameters are opposite the effects of equatorial coordination. Thus, as the number of equatorial nitrogen ligands increases, g‖ decreases and A‖ increases, whereas, axial nitrogen coordination causes g‖ to increase and A‖ to decrease.

Pyridine was added to the sludge HA-Cu complex at M:L ratios of 1:1, 1:2, 1:4, 1:6, 1:30 and 1:60. All py adducts gave anisotropic spectra at room temperature, demonstrating that py had not displaced HA oxygen donor ligands coordinated to Cu^{2+} (Table 8.3). The addition of py at a M:L ratio of 1:1 resulted in the substitution of one equatorial H_2O ligand by py as evidenced by a decrease in g‖ and an increase in A‖ (Table 8.3). The addition of py at a M:L ratio of 1:2 caused g‖ to decrease further (Table 8.3), demonstrating that two equatorially coordinated H_2O ligands were substituted by py. The g‖ value of the 1:2 py adduct was higher than that observed for the 1:2 py adduct of the soil HA-Cu complex (Table 8.1). The higher g‖ value observed for the sludge HA-Cu complex again suggested a strengthening of the axial ligand field of Cu^{2+} due to axial coordination of sludge HA ligands. The 1:4 and 1:6 py adducts gave almost identical g‖ and A‖ values as the 1:2 py adduct (Table 8.3) indicating that no further substitution of H_2O ligands by py had occurred beyond that resulting from the addition of py at a M:L ratio of 1:2. Two H_2O ligands were substituted by py at the Cu^{2+} center when py was added at M:L ratios of 1:2, 1:4 and 1:6, demonstrating that there were two equatorial Cu-HA oxygen bonds in the original sludge HA-Cu complex. When py was added at M:L ratios of 1:30 and 1:60, the g‖ and A‖ values remained essentially the same as in the 1:2, 1:4 and 1:6 py adducts (Table 8.3). In the study of Cu^{2+} binding by a soil HA, the addition of py at similar levels (M:L ratios of 1:25 and 1:50) resulted in the substitution of axial H_2O ligands by py, as evidenced by an increase in g∥ and a decrease in A∥ relative to the 1:2 py adduct. For sludge HA, no such axial substitution of H_2O ligands by py was observed indicating that the axial sites were occupied by HA donor ligands. As mentioned above, the higher g∥ and lower A∥ values observed for the 1:2 py adduct (Table 8.3), as compared to the 1:2 py adduct of the Chalmers soil HA-Cu complex (Table 8.1), also suggested axial coordination of sludge HA ligands. The g∥ and A∥ values reported here for the 1:2 py adduct are almost identical to those of the 1:25 and 1:50 py adducts shown for the Chalmers soil Cu-HA complex. The 1:25 and 1:50 py adducts of the soil HA-Cu complex were shown to have two equatorial sites and two axial sites occupied by py with the remaining two equatorial sites of Cu^{2+} occupied by HA oxygen donor ligands (a CuO_2N_4 chromophore). By analogy, the 1:2 py adduct (and the 1:4, 1:6, 1:30 and 1:60 py adducts) for sludge HA-Cu may also be a CuO_2N_4 chromophore with two of the equatorial sites occupied by py, the remaining two equatorial sites occupied by HA oxygen donor ligands, and the axial sites occupied by sludge HA nitrogen donor ligands. The nitrogen content of the Chicago sludge HA has been shown to be over twice that of the Chalmers soil HA, primarily due to the presence of proteinaceous materials in the sludge HA fraction (Section 6). The interaction of proteinaceous materials present in sludge organic matter with metal ions such as Cu^{2+} has also been demostrated (Section 5). The results of the above studies suggest that the sludge HA donor ligands bound axially in the Cu-HA complex may originate from proteinaceous materials associated with the sludge HA fraction.

The bidentate nitrogen donor ligand 1,10-phenanthroline was also added to the sludge HA-Cu complex at M:L ratios of 1:1 and 1:2. As observed for the py adducts, the 1:1 and 1:2 phen adducts gave anistropic spectra at room temperature demonstrating that the coordinated sludge HA donor ligands were not displaced by phen. The addition of phen at a M:L ratio of 1:1 caused g∥ to decrease (Table 8.3) resulting from equatorial nitrogen coordination. The g∥ and A∥ values of the 1:1 phen adduct were similar to those reported in the literature for various other CuO_2N_2 chromophores (Table 8.2). Two equatorial H_2O ligands were substituted by phen in the 1:1 phen adduct. The Cu^{2+} g∥ value remained essentially the same in the 1:2 phen adduct, a result consistent with and analogous to the 1:4 py adduct. Based on this characterization of the phen adducts, the formation of two equatorial sludge HA-Cu oxygen bonds in the original sludge HA-Cu complex was again indicated.

Another bidentate nitrogen donor, en, was added to the sludge HA-Cu complex at M:L ratios of 1:1 and 1:2. The addition of en at a M:L of 1:1 caused g∥ to decrease (Table 8.3) resulting from the displacement of two equatorial H_2O ligands and the formation of two Cu-nitrogen bonds involving en. The 1:1 en adduct was characterized as having equatorial coordination of two sludge HA oxygen donor and the two nitrogen donor atoms of en. The lower g∥ value of the 1:1 en adduct as compared to $[Cu(en)(H_2O)_2]^{2+}$ (Table 8.2) demonstrated that the ligand field strength of the HA oxygen donor ligands was greater than that of H_2O. As the ligand field strength of the equatorially bound ligands increases, the g∥ value of Cu^{2+} decreases (Vanngard, 1972). The g∥ value of Cu^{2+} continued to decrease in the 1:2 en adduct and was identical to that reported for $[Cu(en)_2]^{2+}$ (Barbucci and Campbell, 1976). The observation of an isotropic spectrum at room temperature (rotating Cu^{2+}) confirmed that $[Cu(en)_2]^{2+}$ had formed. Ethylenediamine, which is a stronger based than py or phen, displaced both coordinated H_2O and HA donor ligands from Cu^{2+}. In the 1:2 en adduct, the strength of the equatorial bonds involving the four nitrogen donor atoms of en appeared to prohibit the axial 'coordination' of HA donor ligands observed in the sludge Cu-HA complex. For Cu^{2+}, as the equatorial bonding becomes stronger, the axial ligands are given further from the metal (Smith, 1970). This phenomena is illustrated by the exceptionally low formation constant for the addition of a third en ligand to Cu^{2+} (log K_1 = 10.55, log K_2 = 9.05 and log K_3 = -1.0, Cotton and Wilkinson, 1972) and explains why Cu^{2+} did not remain bound axially in the 1:2 en adduct.

The formation of CuO_2N_2 chromophores in the 1:1 phen and en adducts required that the two equatorial HA oxygen donor ligands occupy cis positions in the square plane of Cu^{2+}. Such an arrangement of sludge HA oxygen donor ligands would be expected with the formation of a chelate ring in the original sludge HA-Cu complex. Although sterically unfavorable, the formation of a chelate ring involving en or phen could possibly cause trans HA oxygen donor ligands to assume cis positions.

The dipeptide glycylglycine was also added to the sludge HA-Cu complex. At pH 4 (pH of the sludge HA-Cu suspension), Cu^{2+} has been shown to form equatorial coordinate bonds involving the terminal amino group and peptide carbonyl oxygen of glygly (Kim and Martell, 1963). The addition of glygly at a M:L ratio of 1:1 caused $g_\parallel$ to decrease resulting from equatorial coordination. The $g_\parallel$ value of the 1:1 glygly adduct was identical to that observed for the 1:1 py adduct indicating the formation of a CuO_3N chromophore (Table 8.3). The room temperature spectrum of the 1:1 glygly adduct was anisotropic. Thus, it appeared the glygly displaced two equatorial H_2O ligands resulting in the formation of bonds involving the terminal amino group and peptide carbonyl oxygen of glygly, as suggested by Kim and Martell (1963). Thus, proteinaceous materials such as glygly, in addition to those associated with the HA fraction of sludge, were shown to interact with Cu^{2+} bound to HA. These results are consistent with an earlier study (Section 5) where infrared techniques were used to demonstrate that metal ions were bound to proteinaceous materials.

SUMMARY

Electron spin resonance (ESR) spectroscopy was used to study the mechanism of Cu^{2+} binding by humic acid (HA) extracted from a Chalmers silt loam soil and a sewage sludge from Chicago, IL. The soil HA-Cu complex and some adducts with nitrogen donors were examined. The ESR data presented showed that the addition of pyridine (py) and 1,10-phenanthroline (phen) to the soil HA-Cu complex resulted in the substitution of coordinated aquo ligands by the nitrogen donor ligands without displacement of HA oxygen donor ligands also coordinated to the Cu^{2+} center. The addition of a more basic nitrogen donor, ethylenediamie (en), resulted in the displacement from Cu^{2+} of both coordianted aquo ligands and HA oxygen donors, forming $Cu(en)_2^{2+}$. The $g_\parallel$ and $A_\parallel$ values obtained for the py and phen adducts revealed that there were two HA oxygen donors coordinated equatorially to the Cu^{2+} center in the original soil HA-Cu complex. The characterization of the adducts with phen and en was consistent with the formation of a Cu^{2+} chelate in the original soil HA-Cu complex, where the two equatorial soil

HA-Cu oxygen bonds would occupy cis positions. The crystal field strength of soil HA oxygen donor ligands coordinated to the Cu^{2+} center was stronger than that of H_2O. For sludge HA, the Cu^{2+} ion also appeared to form axial bonds with sludge HA nitrogen donor ligands originating from proteinaceous materials associated with the sludge HA fraction. Characterization of an adduct with glycylglycine showed that the dipeptide formed coordinate bonds with Cu^{2+} bound to sludge HA.

Table 8.1. ESR spectral data for a Cu-humic acid (HA) complex and some N adducts at 77°K.

Sample (metal:ligand)	$g_\parallel$	g	$A_\parallel$*	Room temp. spectra	Probable+ chromophore
Cu^{2+}/HA	2.345	2.059	151	non-rotating	CuO_4
Cu^{2+}/HA/py (1:1)	2.29	2.053	174	non-rotating	CuO_3N
Cu^{2+}/HA/py (1:2)	2.25	2.047	197	non-rotating	CuO_2N_2
Cu^{2+}/HA/py (1:4)	2.24	2.031	193	non-rotating	CuO_2N_2
Cu^{2+}/HA/py (1:6)	2.25	2.032	189	non-rotating	CuO_2N_2‡
Cu^{2+}/HA/py (1:25)	2.26	2.024	179	non-rotating	CuO_2N_4
Cu^{2+}/HA/py (1:50)	2.26	2.022	179	non-rotating	CuO_2N_4
Cu^{2+}/HA/phen (1:1)	2.25	2.047	174	non-rotating	CuO_2N_2
Cu^{2+}/HA/phen (1:2)	2.23	2.036	172	non-rotating	CuO_2N_2
Cu^{2+}/HA/en (1:1)	2.25	2.048	179	non-rotating	CuO_2N_2
Cu^{2+}/HA/en (1:2)	2.185	2.033	209	rotating	CuN_4

* Units of 10^{-4} cm^{-1}

+ CuO_2N_4 implies axial positions occupied by nitrogen donors. All other species have axial positions occupied by H_2O.

‡ Partial axial 'corrdination' by py

Table 8.2. ESR spectral data for various Cu (II) complexes in aqueous solutions.

Compound	$g_\parallel$	g	$A_\parallel$*	Chromophore
$Cu\gamma(5\text{-}ClSAL)_2\ 3H_2O^+$	2.35	2.04		CuO_4
$[Cu(lactate)(H_2O)_2]^+$	2.330	2.057		CuO_4
$Cu(malonate)(H_2O)_2$	2.334	2.073	153	CuO_4
$Cu(maleate)(H_2O)_2$	2.354	2.070	144	CuO_4
$[Cu(oxalate)_2]^{2-}$	2.318	2.071	164	CuO_4
$[Cu(aq)]^{2+}$	2.422	2.089	134	CuO_4
$Cu(5\text{-}ClSAL)_2(py)_2^+$	2.26	2.06		CuO_2N_2
Cu(bipy)(phthalate)	2.264	2.071	174	CuO_2N_2
Cu(bipy)(malonate)	2.256	2.069	174	CuO_2N_2
Cu(bipy)(maleate)	2.262	2.074	174	CuO_2N_2
Cu(bipy)(oxalate)	2.258	2.063	183	CuO_2N_2
$[Cu(en)(H_2O)_2]^{2+}$	2.272	2.070	177	CuO_2N_2
$[Cu(bipy)(py)]^{2+}$	2.249	2.069	186	$CuON_3$
$[Cu(2,2\text{-}tri)(H_2O)]^{2+}$	2.226	2.042	190	$CuON_3$
$[Cu(en)_2]^{2+}$	2.200	2.045	200	CuN_4
$[Cu(py)_4]^{2+}$	2.22		180	CuN_4
$[Cu(3\text{-}picoline)_4]^{2+}$	2.21		183	CuN_4

* $A_\parallel$ in units of 10^{-4} cm^{-1}.

+ Powder spectra.

Table 8.3. ESR spectral data for a Cu (II) complex of sludge humic acid (HA) and some adducts with nitrogen donors at 77°K.

Sample (metal:ligand)		$g_{\parallel}$	$A_{\parallel}^{*}$	Room temp spectra	Probable chromophore
Cu^{2+}/HA		2.36	154	non-rotating	CuO_4
Cu^{2+}/HA/py	(1:1)	2.29	176	non-rotating	CuO_3N
Cu^{2+}/HA/py	(1:2)	2.27	176	non-rotating	CuO_2N_2
Cu^{2+}/HA/py	(1:4)	2.27	175	non-rotating	CuO_2N_2
Cu^{2+}/HA/py	(1:6)	2.27	176	non-rotating	CuO_2N_2
Cu^{2+}/HA/py	(1:30)	2.26	178	non-rotating	CuO_2N_2
Cu^{2+}/HA/py	(1:60)	2.26	175	non-rotating	CuO_2N_2
Cu^{2+}/HA/phen	(1:1)	2.25	179	non-rotating	CuO_2N_2
Cu^{2+}/HA/phen	(1:2)	2.26	166	non-rotating	CuO_2N_2
Cu^{2+}/HA/en	(1:1)	2.25	181	non-rotating	CuO_2N_2
Cu^{2+}/HA/en	(1:2)	2.20	193	rotating	CuN_4
Cu^{2+}/HA/glygly	(1:1)	2.29	174	non-rotating	CuO_3N

* Units of 10^{-4} cm^{-1}

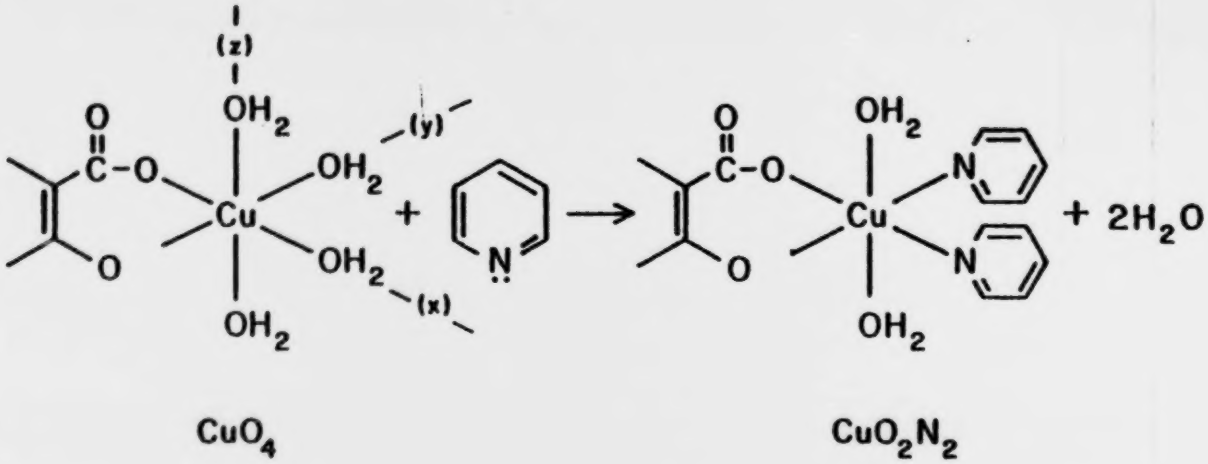


Figure 8.1. The selective displacement of equatorially coordinated H_2O ligands, at Cu:py ratios of 1:2 and 1:4, resulting in the formation of a CuO_2N_2 chromophore. At Cu:py ratios of 1:25 and 1:50, the axial sites (z axis) were also occupied by py, forming a CuO_2N_4 chromophore. The room temperature spectrum of each complex was anisotropic (non-rotating Cu^{2+}) demonstrating that HA oxygen donor ligands coordinated to Cu^{2+} had not been displaced.

SECTION 9

EFFECT OF pH AND REDOX ON PREDICTED HEAVY METAL-CHELATE EQUILIBRIA IN SOILS

INTRODUCTION

A major concern in applying municipal and industrial wastes to agricultural land is the enrichment of soils with metals such as Cu, Zn, Cd, Pb, and Ni. The impact of sewage sludge-borne metals on soils and crops has been summarized in recent reports (CAST, 1976; 1980) which emphasized the scarcity of information available on the chemistry of metals in soils and on suitability of present soil testing procedures to evaluate the availability of metals to crops.

Chelating agents are often used as extractants for assessing plant-available trace metals. In their review of micronutrient soil test Cox and Kamprath (1972) indicate that EDTA has been used for Cu and Mn, and DTPA for Cu, Zn, Mn, and Fe. Although most soil tests are designed to detect metal deficiencies, the DTPA soil test has been utilized to assess the plant availability of metals added to soils at elevated levels in sewage sludges (Channey *et al.*, 1976; Bingham *et al.*, 1976; Kelling *et al.*, 1977). In a greenhouse experiment using sewage sludge enriched with $CdSO_4$, a significant correlation (r = 0.96) was found between DTPA-extractable Cd and Cd in corn seedlings (Street *et al.*, 1977).

Chelating agents have also been used to study metal ion activities in soils. Stability diagrams developed by Lindsay and Norvell (1969) and Norvell (1972) indicate that equilibrium relationships of metal chelates in soils depend upon: (i) the ligand used, (ii) the solid phase(s) controlling metal solubilities, and (iii) the pH of the system. Based on theoretical calculations, competitive chelate equilibria have been used to determine Cu^{2+}, Zn^{2+}, and Fe^{3+} ion activities in soils (Norvell and Lindsay, 1969; Lindsay and Norvell, 1969; Norvell, 1971). This approach is useful because the solubility of many metals is below the detection limits of most commonly used analytical methods, especially in alkaline systems. In soil studies, EDTA- and DTPA-extractable Cu, Zn, Pb, and Cd have been monitored in sewage sludge-soil systems to evaluate changes in metal forms during laboratory incubation studies (Bloomfield and Prduen, 1975; Slviera and Sommers, 1977). Similarly, EDTA is useful for extracting metal carbonates, but not metal sulfides from sewage sludges (Stover *et al.*, 1976).

Even though considerable work has been reported on the use of chelates to extract metals such as Cd, Pb, and Ni in soils or soil-waste systems, no systematic evaluation of chelate-metal equilibrium relationships has been made. The objectives of this section were to develop stability diagrams for 5 chelating agents and 12 metals as a function of pH and redox potential (pe + pH) in order to select those chelating agents that are most useful for extracting metals from soils and for studying the activity of metal ions in different soil environments.

MATERIALS AND METHODS

The ligands used in this study were CDTA (cyclohexane-1,2-diaminetetra-acetic acid), DTPA (diethylenediaminepentaacetic acid), EDTA (ethylenediamine-tetraacetic acid), EGTA [ethyleneglycolbis (ethylamine) tetraacetic acid], and HEDTA (hydroxyethylenediaminetriacetic acid). The metals included H^+, Al^{3+}, Fe^{3+}, Fe^{2+}, Mn^{2+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, and Ni^{2+}. Stability constants for metal-ligand reactions were obtained from Martell and Smith (1974) except for the Fe(III) L species of DTPA which was obtained from Bottari and Anderegg (1967). The equilibrium constants were all adjusted to $K^c_{0.1}$ and $K^m_{0.01}$ using the Davies equation and procedures outlined by Lindsay (1979). The stability constants obtained from the literature ($K^c_{0.1}$) and corrected for use in this study ($K^m_{0.01}$) are summarized in Table 9.1.

Equilibrium constants (K^o) for the solid phases used to control metal solubility in soils are summarized in Table 9.2 and were obtained from Lindsay (1979). These constants were also converted to $K^m_{0.01}$ with the Davies equation for use in this study. Use of mixed constants at 0.01 ionic strength for metal-chelate studies simplifies the application of theoretical mole fraction diagrams to experimental data obtained by measuring concentrations, rather than activities, of metal ions. In addition, most non-saline soils are readily adjusted in the laboratory to an ionic strength of 0.01.

The approach used in developing stability diagrams for metal-ligand systems is similar to that used by Lindsay and Norvell (1969). Concentrations and activities are denoted by brackets and parenthesis, respectively, in the following equations. The total ligand can be partitioned as follows:

$$L_T = L + \Sigma H_nL + \Sigma Fe(III)XL + \Sigma Fe(II)XL + \Sigma AlXL + \Sigma MnXL + \Sigma CaXL + \Sigma MgXL + \Sigma ZnXL + \Sigma CuXL + \Sigma CdXL + \Sigma PbXL + \Sigma NiXL + \Sigma MXL \quad (1)$$

where L_T = total ligand, L = free ligand, ΣH_nL = sum of protonated ligand species, Fe(III)XL = sum of Fe(III) ligand species and so forth. Charges on individual species have been omitted for simplicity. The MXL term refers to other metals capable of forming chelates with each ligand. This term was not included in the mole fraction diagrams developed since the above 12 metal should account for nearly all metals in typical soils. The X in the above terms signifies the inclusion of H^+ or OH^- in some metal chelates. Only the oxidation state of Fe is specified because the oxidation state of II was used for all other metals, except for H^+ and Al^{3+}. All ligand species listed in Table 9.1 were included in calculating the mole fractions (MF) for the various metals, i.e., the proportion of the total ligand associated with each metal. This approach allows the results to be used at any total ligand concentration since multiplication of the mole fraction for a given metal or metal-ligand species and the total ligand concentration gives the concentration of that species.

Mole fractions are readily calculated from Equation 1, since the free ligand (L) can be factored from each term on the right-hand side of this equation. Using EDTA as an example, the Fe(III)XL term includes the following:

$$\Sigma Fe(III)XL = Fe(III)L^- + Fe(III)HL^o + Fe(III)OHL^{2-} \quad (2)$$

where L signifies EDTA. Each term in Equation 2 can be expressed in terms of an equilibrium constant, Fe(III) concentration, and H^+. Using the $K^m{}_{0.01}$ values from Table 9.1 for EDTA,

$$\Sigma Fe(III)XL = 10^{26.61}\,[Fe(III)]\,[L^{4-}] + 10^{28.08}[Fe(III)]\,[L^{4-}]\,(H^+) + \frac{10^{18.80}\,[Fe(III)]\,[L^{4-}]}{(H^+)} \quad (3)$$

The Fe(III) concentration can be calculated from Equation 6 of Table 9.2.

$$[Fe(III)] = 10^{3.10}\,(H^+)^3 \quad (4)$$

Substitution of Equation 4 into 3 yields

$$\Sigma Fe(III)XL = 10^{29.72}[L^{4-}](H^+)^3 + 10^{31.19}\,[L^{4-}](H^+)^4 + 10^{21.91}\,[L^{4-}](H^+)^2 \quad (5)$$

The redox parameter, pe + pH, was used rather than E_h to simplify calculation of metal solubilities and subsequent metal-ligand concentrations. For example, the Fe(II)XL term of Equation 1 includes

$$\Sigma Fe(II)XL = Fe(II)L^{2-} + FeHL^- \quad (6)$$

which becomes

$$\Sigma Fe(II)XL = 10^{15.34}[Fe(II)]\,[L^{4-}] + 10^{18.36}[Fe(II)]\,[L^{4-}]\,(H^+) \quad (7)$$

If $Fe(OH)_3$ (soil) controls Fe(II), then

$$[Fe(II)] = 15.92\,(H^+)^3(e^-) \quad (8)$$

Substitution of 8 into 7 yields

$$\Sigma Fe(II)XL = 10^{31.26}\,[L^{4-}]\,(H^+)^3(e^-) + 10^{34.28}\,[L^{4-}]\,(H^+)^4(e^-) \quad (9)$$

Thus, redox reactions were treated in the same manner as metal solubility or chelate stability relationships which simplified calculating the mole fraction of each species as a function of both pH and redox. The above example assumes that $Fe(OH)_3$ (soil) controls Fe^{2+}. From a theoretical standpoint it is often advantageous to express redox in terms of pe + pH (Lindsay, 1979). From Equation 8, Fe^{2+} is defined by

$$[\log Fe(II)] = 15.92 - 2pH - (pe + pH) \qquad (10)$$

The pe + pH parameter is useful because it separates the protons associated with a redox reaction from the protons involved in the acid-base portion of the reaction.

After all terms in Equation 1 have been expressed in terms of K, metal ion concentration, pH and pe + pH, the mole fraction can be calculated. Using Fe(III)XL as an example (see equation 5),

$$MF = \frac{\Sigma Fe(III)XL}{L_T} = \frac{L^{4-}[10^{29.72}(H^+)^3 + 10^{31.19}(H^+)^4 + 10^{21.91}(H^+)^2]}{L^{4-}\ (TOT)} \qquad (11)$$

where TOT signifies all terms in Equation 1 expressed in the same manner as Equations 5 and 9 for all metal-ligand species after factoring out L^{4-}. Since L^{4-} is common to both numerator and denominator, it cancels out and the mole fraction can be calculated for any fixed pH and pe + pH.

In all mole fraction diagrams developed, total SO_4^{2-} was set at 10^{-3} M and CO_2 partial pressure at 0.003 atm. to represent conditions most commonly expected in soils. All calculations were performed using computer programs written in Basic-Plus.

Solubility relationships of the 11 metal ions included in this study are shown in Fig. 9.1. The assumptions used for Fe^{3+}, Al^{3+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+} are similar to those employed by Norvell (1972). That is, Fe^{3+} and Al^{3+} are controlled by their respective hydrous oxides, while Ca^{2+} is buffered at $10^{-2.32}$ M until $CaCO_3$ precipitates at an alkaline pH. The Mg^{2+} concentration was set at 10^{-3} M and then was limited to 10 times the Ca^{2+} at pH values where $CaCO_3$ precipitates. Soil solid phases, as yet unidentified, were used to control Zn^{2+}, (Lindsay and Norvell, 1969) and Cu^{2+} (Norvell and Lindsay, 1969). Manganese solubility was governed by pyrolusite (MnO_2). Major problems were encountered in describing Cd, Pb, and Ni solubility relationships in soils. For Pb, $Pb_3(PO_4)_2$ was chosen as an appropriate solid phase based on theoretical considerations. Collaborating data has been presented by Sandtillan-Medrano and Jurinak (1975). Either varascite or octacalcium phosphate (OCP) were selected to control P solubilities whichever maintained the lowest level of P. Alternatively, the diagrams could have been constructed using an appropriate function for soluble P concentration, if sorption reactions rather than variscite and OCP were selected to control P solubility in soils. An additional Pb^{2+} solid phase that could be included is $Pb_5(PO_4)_3Cl$, which would maintain a somewhat lower Pb^{2+} concentration than $Pb_3(PO_4)_2$ at typical Cl^- concentrations found in soils. Further studies are needed to determine if $Pb_5(PO_4)_3Cl$ can indeed control Pb^{2+} in soils. With respect to Cd^{2+}, an upper limit of 10^{-7} M Cd^{2+} was established at pH values below those allowing precipitation of $CdCO_3$. This limit was based on the data of Street *et al.*, (1977), even though Santillan-Medrano and Jurinak (1975) found Cd^{2+} activities approaching 10^{-3}

M in soils of pH 6.0 - 6.5 and suggested the formation of either $Cd_3(PO_4)_2$ or $CdCO_3$.

Great uncertainties are involved in estimating Ni^{2+} concentrations in soil solutions. Evaluation of $Ni(OH)_2$, NiO, and $Ni_3(PO_4)_2$ indicated that soluble Ni^{2+} concentrations would exceed 10^{-2} M in acid soils if these solids were present. Obviously such Ni^{2+} levels are too high to occur in most soils. An arbitrary limit of 10^{-5} M Ni^{2+} was thus set for acid conditions and $NiCO_3$ controlled Ni^{2+} above pH 7.6. The choice of 10^{-5} M Ni^{2+} is consistent with observations of 2 mg/l of soluble Ni^{2+} (3×10^{-5} M) as found in saturation extracts from an acid soil obtained beneath a sludge drying lagoon (Bradford et al., 1975).

Under reducing conditions, additional solid phases were included; $FeCO_3$, FeS_2, $MnCO_3$, MnS, CdS, Cu_2S, ZnS, NiS, and PbS. Based on theoretical considerations, Lindsay (1979) concluded that ZnS, NiS, and MnS would not form in soils unless there is sufficient SO_4^{2-} to first precipitate all labile Fe as FeS_2. Typically, SO_4^{2-} would be exhausted prior to the precipitation of all Fe as FeS_2, so that the remaining Fe^{3+} oxides would continue to control Fe^{2+}. Since sewage sludges contain appreciably more S than soils, stability diagrams were developed to reflect both the presence and absence of FeS_2, ZnS, NiS, and MnS. It was assumed that CdS, Cu_2S and PbS precipitated in all systems at the appropriate pe and pH levels. Since total Fe nearly always exceeds total P in soils there would be insufficient P to precipitate all Fe as $Fe_3(PO_4)_2 \, 8H_2O$ (vivianite). A maximum concentration of 10^{-2} M was established for Mn^{2+} and Fe^{2+} on the basis that $MnCO_3$ and $FeCO_3$ would form and limit their respective solubilities.

For simplicity, the charge on most metal ions is omitted in the discussion that follows.

RESULTS

The stability diagrams developed herein mainly concern the competitive equilibria between the principal indigenous soil metals including Fe(III), Fe(II), Al, Mn, Ca, Mg and metals added to soils through waste applications which include Cu, Zn, Pb, Cd and Ni. The results are divided into three sections: 1) Ni chelate relationships, 2) the effect of pH on metal chelate stabilities at pe + pH = 17 to approximate the redox status of well-drained soils and 3) the effect of redox on metal chelate stability at pH 6.5.

Ni chelate Relationships

A major problem encountered was evaluating Ni chelate relationships due to the uncertainty of the solid phases controlling Ni solubility in soils. Even with a maximum solubility limit of Ni^{2+} set at 10^{-5} M, Ni ligand species dominated the stability diagrams at pH > 5.0 for all ligands, except for EGTA. In acid soils where Cd and Pb were limitedd to 10^{-7} and 10^{-5} M, respectively, the pH at which Ni becomes the major species is illustrated in Fig. 9.2 for DTPA at Ni levels of 10^{-5}, 10^{-6}, and 10^{-7} M. Diagrams for DTPA, CDTA, and HEDTA were similar to Fig. 9.2 but exhibit differences in the pH values for > 95% saturation of the ligand. Even at 10^{-7} M, Ni DTPA constitutes the dominant species above pH 6.5. In essence, EDTA, CDTA, DTPA, and HEDTA should extract most of the labile Ni from soils. Extraction of soils or wastes with an alkaline solution (pH 7-9) of the above ligands would insure even greater recovery of Ni. Except for EGTA, Ni was excluded in developing the chelate stability diagrams that follow because of the lack of reliable information on Ni solubility in soils. Furthermore, previous results indicate that Ni normally does not dominante the chelates studied (Norvell and Lindsay, 1969; 1972).

Stability Diagrams at Varying pH

Stability diagrams showing the effect of pH on metals associated with various chelating agents were developed for slightly reduced soils of pe + pH = 17.

DTPA--

The predominant species of DTPA below pH 6.2 is Fe(III)-DTPA (Fig. 9.3). From pH 5 to 7, significant amounts of Cu-, Zn-, Pb-, Cd-, and Mn-DTPA are also present. Above pH 6.5, Cd-DTPA is the predominant species. Since the DTPA soil test is buffered at pH 7.3, this procedure should extract appreciable amounts of Cd from sludges and soils. Several reports substantiate the extractability of Cd by DTPA. The present findings suggest that extractions with DTPA buffered at pH 6.0 to 6.5 could be used to assess Fe(III), Cu, Zn, Pb, Cd, and Mn in soils. However, at this pH, significant amounts of Fe would be extracted. Also $CaCO_3$ would dissolve from calcareous soils in this pH range causing an increase in both pH and Ca.

EDTA--

In contrast to DTPA, both Fe(III)- and Pb-EDTA predominate in acid systems (Fig. 9.4). Fe(III)-EDTA is a major component below pH 5.2 and Pb predominates above this pH. Cd-EDTA replaces Pb-EDTA at pH > 7.7 but both metals are important in neutral and calcareous systems. The main occurrence of Zn-, Cu-, and Mn-EDTA is from pH 5.0 to 7.0, but they are always < 10% of the total ligand. In calcareous systems, Ca- and Mg-EDTA species are relatively unimportant. Alkaline solutions of EDTA should be effective in extracting Cd and Pb from soils. The $(NH_4)_2CO_3$-EDTA reagent of Trierweiler and Lindsay (1969) should be useful for this purpose since adding CO_3^{2-} depresses Ca and allows a greater concentration of free ligand to combine with Zn and Cu. Alternatively, EDTA in the pH region of 6.5-7.5 should be effective in removing Pb, Cd, Zn, and Cu from soils.

CDTA--

The equilibrium relationships for CDTA are shown in Fig. 9.5. For CDTA, Fe(III) species dominate the acid region to pH 6.9 whereupon Cd-EDTA becomes predominant. The Pb-CDTA species are of secondary importance from pH 5 to 9, reaching a maximum of about 10% of the ligand at pH 7.4. Of lesser consequence are Zn, Cu, and Mn which constitute about 1% of the ligand at pH 6.9. CDTA is equally effective as EDTA and DTPA in chelating Cd in calcareous systems but it is not as effective in acid systems.

EGTA--

EGTA differs from the 3 previously discussed ligands in that Fe(III) is not a major species even in acid systems (Fig. 9.6). Cu-EGTA dominates below pH 5.7 while Cd-EGTA is the main species above this pH. Nickel is included in this diagram since Ni-EGTA is not as stable as Ni chelates of EDTA, CDTA, DTPA, or HEDTA (Ni is omitted from Fig. 9.3, 9.4, 9.5, and 9.7 as previously mentioned). Ni-EGTA is > 1% of the ligand from pH 5.0 to 9.0. In the pH region of 5.0 to 6.0, Cu, Cd, Ni, Pb, and Mn species constitute a significant percentage of the total ligand. Based on these results EGTA offers considerable potential in acid soils for studying these metals. A significant advantage of EGTA over EDTA, DTPA, or CDTA is the low stability of the Fe(III) and Al chelates which would avoid excessive amounts of Fe(III) and Al being extracted from acid systems. EGTA would not be a suitable soil extractant for Zn because of the low stability of Zn-EGTA.

HEDTA--

The behavior of HEDTA and EGTA are somewhat similar in acid systems in that Cu is the predominate species present (Fig. 9.7). However, Pb-HEDTA is the main species above pH 5.2 while Cd-HEDTA approaches 30% of the ligand above pH 7.9. From pH 5.5 to 7.0, Pb, Cu, and Zn all constitute a significant percentage of the total ligand. As with EGTA, both Fe(III) and Al are relatively unimportant over the pH range shown. Major dissimilarities with EGTA are the greater stability of Zn-HEDTA and the decreased stability of Mn-HEDTA. In Calcareous soils Ca and Mg compete favorably with Zn and Cu for HEDTA.

Effect of Redox on Metal Chelate Stability

Many organic wastes added to soils have been previously treated by anaerobic digestion, particularly sewage sludges. Many solid phases present in anaerobic environments are not stable in moderately well-oxidized soils, consequently chemical transformations will occur. To this end, stability diagrams were developed at fixed pH and varying redox in terms of pe + pH. Diagrams were developed for EDTA, DTPA, EGTA and HEDTA. A pH of 6.5 was selected because many anaerobic systems tend to approach this pH value.

The same solid phases discussed previously were used to control metal solubilities (Table 9.2). Stability diagrams were developed for two situations: (i) when sufficient S was present to precipitate Cu_2S, PbS and CdS but not sufficient S to precipitate the sulfides of Fe(II), Zn, and Mn; (ii) when sufficient S was present to precipitate all six metals as sulfides. In the first case, Fe^{2+} and Mn^{2+} were limited by their respective carbonates at pH 6.5 (Lindsay, 1979). In the second case, metal sulfides controlled metal solubility. The rationale for presenting both situations is based on the fact that many soils do not contain a sufficient amount of S to precipitate all the Fe and Mn present. In contrast, some environments may receive a continual supply of S resulting in the ultimate precipitation of most metals as sulfides (e.g., anaerobic digesters or lake sediments). Vivianite, $Fe_3(PO_4)_2\ 8H_2O$ was not used as a solid phase controlling Fe(II) because, in most systems, there is insufficient P to combine with all the Fe present.

Again, the diagrams presented here do not include Ni^{2+} for reasons discussed earlier. In highly oxidized soils (pe + pH > 18), Mn^{2+} solubility is depressed by MnO_2 and does not compete effectively for most chelating ligands. In essence, this increases the mole fractions of all other metal ligands. In oxidizing environments, the major species present are Cd-EGTA, Cd-DTPA, Pb-EDTA, and Pb-HEDTA.

In the pe + pH region of 6 to 16, $MnCO_3$ controls Mn^{2+} at $10^{-3.24}$ M. At this Mn^{2+} concentration, Mn is the predominant metal on DTPA (Fig. 9.8) and EDTA (Fig. 9.9). Under reduced conditions, CO_2 concentrations will very likely increase resulting in depressed Mn^{2+} levels and thus, a decrease in the fraction of the ligand associated with Mn. A significant amount of Cd-DTPA is also present in this redox region. Cadmium dominates the EGTA diagram (Fig. 9.10) while Mn and Pb are of equal importance for HEDTA (Fig. 9.11). Of the 4 ligands discussed, both DTPA and HEDTA should be good extractants for Mn, Cu, Zn, Pb, and Cd in soils at intermediate redox levels.

Below pe + pH of 7, significant changes occur in the metal-ligand stabilities due to the precipitation of CdS, PbS, and Cu_2S, when there is insufficient as SO_4^{2-} to precipitate all soil Fe as FeS_2. As Cd, Pb and Cu precipitate as sulfides, their concentrations in solution drops to extremely low levels allowing either Mn or Fe(II) to dominate the ligand (Fig. 9.8 to 9.11). The carbonates of Fe(II) and Mn are the solid phases preesnt supporting concentrations of $10^{-3.4}$ and $10^{-3.24}$ M, respectively. Due to these concentration differences and the stability constants for Fe(II) and Mn(II), Fe(II) is the predominant species for DTPA (Fig. 9.8), EDTA (Fig. 9.9), and HEDTA (Fig. 9.11). For EGTA, the proportion of chelated Mn exceeds that of Fe(II) (Fig. 9.10). However, both Fe(II) and Mn(II) constitute 10% of EDTA and EGTA and 1% of DTPA and HEDTA. The only other major metal interacting with these ligands is Ca for EGTA. Even though Zn did not precipitate as ZnS it was a relatively minor species for all ligands because of the greater concentrations of Fe(II) and Mn(II) in strongly reducing systems.

Stability diagrams were developed for ligands where the precipitation of Cu_2S, CdS, PbS, FeS_2, ZnS, and MnS occurred. Only the results for DTPA are shown to illustrate the salient features of such systems (Fig. 9.12).

The major differences between DTPA relationships for limited (Fig. 9.9) and excess (Fig. 9.12) SO_4^{2-}-concentrations occur at a pe + pH of < 5. As shown in Fig. 9.9, Fe(II) is the major ligand species until the precipitation of FeS_2, whereupon Mn becomes an important species (Fig. 9.12). Following MnS precipitation, Ca becomes the predominant metal on the ligand. Also, Al only appears on the ligands under the highly reduced conditions needed for MnS precipitation. Even though Zn was not a major ligand species, the formation of ZnS results in its disappearance from the ligand. A detailed presentation of the chemical reactions occurring under highly reduced conditions are beyond the scope of this discussion.

DISCUSSION

The stability diagrams developed herein are based on equilibrium calculations. Even though thermodynamic equilibrium may never be attained in many soil systems, such calculations show the potential reactions between various ligands and metals in soils. In soils treated with large amounts of sewage sludge or other metal containing wastes, the actual conditions are undoubtedly more complex but the equilibrium relationships of added chelating agents remain valid.

The chelating agents studied can be ranked according to their ability to extract metals from soils at pH's of 5.5, 6.5, and 7.5 (i.e., pH values typical of acid, neutral and calcareous soils). Only those metals constituting > 1% of the ligand at pe + pH of 17 are included (Fe signifies Fe(III)).

	pH 5.5	pH 6.5	pH 7.5
DTPA	Fe>Cu>Zn>Pb>Mn	Cd>Cu>Zn>Fe>Pb>Mn	Cd>Pb
EDTA	Pb>Fe>Zn>Cu>Mn	Pb>Zn>Cd>Fe>Cu>Mn	Pb>Cd>Ca
CDTA	Fe	Fe>Pb>Cd>Mn>Zn>Cu	Cd>Pb>Fe
EGTA	Cu>Pb>Cd>Mn>Ni>Ca	Cd>Ni>Ca>Cu	Cd>Ni>Ca
HEDTA	Pb>Cu>Zn	Pb>Cu>Zn	Pb>Cd>Cu>Zn>Ca

When pH was fixed at 6.5 and pe + pH was varied to simulate soils that are strongly reduced (pe + pH of 1 to 7), moderately reduced (pe + pH of 7 to 17), and highly oxidized (pe + pH > 17), the following stability trends were found:

	pe + pH 1-7	pe + pH 7-17	pe + pH > 17
DTPA	Fe(II) > Mn > Zn	Mn > Cd > Cu > Zn	Cd > Cu > Zn > Fe(III)
EDTA	Fe(II) > Mn > Zn > Ca	Mn > Pb > Zn > Cd	Pb > Zn > Cd > Fe(III)
EGTA	Mn > Fe(II) > Ni > Ca	Mn > Cd > Ni > Ca	Cd > Ni > Ca > Cu
HEDTA	Fe(II) > Mn > Zn	Mn > Pb > Cu > Zn	Pb > Cu > Zn

Except for EGTA, Ni was eliminated from the above comparisons because of the uncertainty regarding an appropriate solid phase for controlling Ni solubility in soils. All of the ligands considered appear to be effective reagents for removal of Cd, Pb, and Ni from neutral and calcareous systems. In acid systems, HEDTA, EDTA, and EGTA should be evaluated as potential metal extractants.

The mole fraction vs. pe + pH diagrams aid in interpretation of experiments where anaerobic sewage sludge is incubated in soils. For example, Bloomfield and Pruden (1971) found that EDTA-extractable Pb, Zn, Ni, and Cd increased during a 3-month incubation. These results are consistent with the hypothesis that metal sulfides were originally present in the sludge and subsequently oxidized to more soluble forms. The extractability of Cu, Zn, Cd, and Pb with DTPA changes during incubation of sludge-soil (Silviera and Sommers, 1977). However, changes in extractability of a metal is also affected by changes in the concentration of competing ions. An excellent example is the change in Zn on a ligand with changes in pe + pH even though Zn^{2+} is controlled by the same solid phase throughout the entire pe + pH range (Fig. 8-12). The first increase in Zn ligand species is caused by the oxidation of Fe(II) to Fe(III) and precipitation of $Fe(OH)_3$ (soil) while the second increase is caused by the precipitation of MnO_2. Thus, if the extractability of a metal is used as a tool to evaluate metal transformations in soils, the soil extract should be analyzed for all metals capable of interacting with the ligand so the effect of competing ions can be evaluated. Since the concentration of most heavy metals in soil extracts, such as 0.01 M $CaCl_2$, are generally insignificant in the absence of a chelating agent, any metal extracted is most likely associated with the chelating agent. In reduced systems, however, appreciable concentrations of soluble Fe(II) and Mn are present and chelating agents, such as organic acids, may be synthesized by microbes. Under these conditions, metal solubility should be assessed in the presence and absence of the added ligand to evaluate metal-chelate equilibria. In addition, the concentration of other ions capable of complexing metals (e.g., HCO_3^-, CO_3^{2-}, $H_2PO_4^-$, Cl^-, etc.) must be known to calculate the free metal concentration.

The metal-chelate equilibrium relationships studied are amenable to predicting the fate of micronutrients, mainly Cu and Zn, added to paddy soils. The diagrams shown for DTPA (Fig. 9.8) and EDTA (Fig. 9.9) indicate that both Cu and Zn chelates will dissociate to a large extent in reduced systems. This hypothesis has been confirmed qualitatively in recent studies by Reddy and Patrick (1977). Their results could not be compared to the predicted relationships reported herein, due to uncertainties in the partial pressure of CO_2, which greatly affects the amount of Mn that is chelated.

The stability diagrams apply directly to equilibrium systems where there is a sufficient concentration of all metals in the soil to obey the solubility relationships of the solid phases used. Often times when soils are extracted with a chelating agent, the total metal concentration present in the soil-extractant system is inadequate to approach the level predicted by the diagrams. For example, if 10^{-6} moles of DTPA at pH 7.3 are reacted with 1 g of soil containing 1 ppm Cd (10^{-8} moles), Fig. 9.3 would indicate that 10^{-6} moles of Cd would be in the extract. Obviously, this cannot be the case since only 10^{-8} moles of Cd are present in the entire system. However, Fig. 9.3 also indicates that upon removal of Cd from the soil, the mole fractions of Pb, Zn, and Mn will increase to compensate for the lack of Cd in the system. Thus, the diagrams can be used to show the ability of different metals to compete for any of the ligands even if the preferred metal is not present at a sufficient concentration to reach the equilibrium level shown in the diagram. In such cases, all metals constituting a lower mole fraction will become increasingly important when the preferred metal is limiting.

The DTPA soil test has been developed to assess Cu, Zn, Mn and Fe deficiencies in calcareous soils (Norvell and Lindsay, 1978). Numerous researchers are currently using this procedure to evaluate Ni, Cd, and Pb in soils treated with sewage sludge. Based on the equilibrium relationships shown in Fig. 9.2, the DTPA soil test has a sound theoretical basis for extracting Cd, Pb, and Ni. This study also demonstrates that several other ligands (e.g., EGTA, HEDTA, and EDTA) could be used to extract metals from soils.

Competition between metal ions for a ligand can be used to evaluate the activity of metals in soil systems. Inspection of the diagrams developed indicates which metals and ligands can be employed in such studies. Furthermore, a given ligand-metal combination may not be appropriate over a wide range of pH or pe + pH. The theoretical considerations developed herein are useful in selecting ligands and conditions for assessing plant available metals in soils, studying metal solubilities in soils, and evaluating critical concentrations of metals for plant uptake.

Table 9.1. Stability constants ($K^c_{.1}$) at 25°C for metal-ligand reactions selected from Martell and Smith (1974) and corrected to mixed constants ($K^m_{.01}$)

	HEDTA		EGTA		EDTA		DTPA		CDTA	
	$K^c_{.1}$	$K^m_{.01}$	$K^c_{.1}$	$K^m_{.01}$	$K^c_{.1}$	$K^m_{.01}$	$K^c_{.1}$	$K^m_{.01}$	$K^c_{.1}$	$K^m_{.01}$
H + L = HL	9.81	10.25	9.40	9.98	10.17	10.75	10.45	11.16	12.3	12.88
2H + L = H_2L	15.18	15.94	18.18	19.20	16.28	17.30	18.98	20.27	18.42	19.44
3H + L = H_3L	17.78	18.71	20.84	22.18	18.96	20.30	23.26	24.99	21.95*	23.29
4H + L = H_4L	-	-	22.84	24.35	20.96	22.47	25.91	27.96	24.37*	25.88
5H + L = H_5L	-	-	-	-	-	-	27.73	29.95	-	-
Ca + L = CaL	8.2	9.01	10.86	11.93	10.61	11.68	10.75	12.09	13.15	14.22
Ca + H + L = CaHL	-	-	14.65*	15.94	13.79*	15.18	16.86	18.64	-	-
2Ca + L = Ca_2L	-	-	-	-	-	-	12.35	14.50	-	-
Mg + L = MgL	7.0	7.81	5.28	6.35	8.83	9.90	9.34	10.68	11.07	12.14
Mg + H + L = MgHL	-	-	12.90a	14.29	12.68*	14.07	16.19	17.97	-	-
Fe^{2+} + L = FeL	12.2	13.01	11.80	12.87	14.27	15.34	16.40	17.74	18.90	19.97
Fe^{2+} + H + L = FeHL	14.95*	15.93	16.10*	17.49	16.97	18.36	21.70	23.48	21.70	23.09
Fe^{2+} + L = FeOHL + H	3.23	-	-	-	5.20*	-	7.41	-	-	-
$2Fe^{2+}$ + L = Fe_2L	-	-	-	-	-	-	19.38*	21.53	-	-

Table 9.1. Continued.

	HEDTA		EGTA		EDTA		DTPA		CDTA	
	$K^c_{.1}$	$K^m_{.01}$	$K^c_{.1}$	$K^m_{.01}$	$K^c_{.1}$	$K^m_{.01}$	$K^c_{.1}$	$K^m_{.01}$	$K^c_{.1}$	$K^m_{.01}$
$Fe^{3+} + L = FeL$	19.8	21.00	20.5	22.11	25.0	26.61	27.3$^+$	29.3	30.0	31.61
$Fe^{3+} + H + L = FeHL$	-	-	-	-	26.3*	28.08	30.86	33.18	-	-
$Fe^{3+} + L = FeOHL + H$	15.92	16.95	-	-	17.51*	18.80	17.42	18.98	20.4*	21.69
$Fe^{3+} + L = Fe(OH)_2L + 2H$	6.90	-	-	-	8.10*	-	-	-	-	-
$Zn + L = ZnL$	14.6	15.41	12.60	13.67	16.44	17.51	18.29	19.63	19.35	20.42
$Zn + H + L = ZnHL$	-	-	17.56*	18.95	19.44	20.83	23.89	25.67	22.25*	23.64
$2Zn + L = Zn_2L$	-	-	15.9*	17.51	-	-	22.77	24.92	-	-
$Cu + L = CuL$	17.50	18.31	17.57	18.64	18.70	19.77	21.38	22.72	21.92	22.99
$Cu + H + L = CuHL$	19.92	20.90	21.85	23.24	21.70	23.09	26.19	27.97	25.02*	26.41
$2Cu + L = Cu_2L$	-	-	21.88	23.49	-	-	28.17	30.32	-	-
$Mn + L = MnL$	10.8	11.61	12.18	13.25	13.81	14.88	15.51	16.85	17.43	18.50
$Mn + H + L = MnHl$	-	-	16.28*	17.67	16.91	18.30	19.91	21.69	20.23*	21.62
$2Mn + L = Mn_2L$	-	-	-	-	-	-	17.60*	19.75	-	-
$Cd + L = CdL$	13.1	13.91	16.5	17.57	16.36	17.43	19.0	20.34	19.84	20.91
$Cd + H + L = CdHL$	-	-	19.97*	21.36	19.26	20.65	23.17	24.95	22.84*	24.23
$2Cd + L = Cd_2L$	-	-	-	-	-	-	21.30	23.45	-	-

Table 9.1. Continued.

	HEDTA		EGTA		EDTA		DTPA		CDTA	
	$K^c_{.1}$	$K^m_{.01}$	$K^c_{.1}$	$K^m_{.01}$	$K^c_{.1}$	$K^m_{.01}$	$K^c_{.1}$	$K^m_{.01}$	$K^c_{.1}$	$K^m_{.01}$
$Pb + L = PbL$	15.5	16.31	14.54	15.61	17.88	18.95	18.66	20.00	20.24	21.31
$Pb + H + L = PbHL$	-	-	19.70*	21.09	20.68*	22.07	23.18	24.96	23.04*	24.43
$2Pb + L = Pb_2L$	-	-	19.14*	20.75	-	-	22.07	24.22	-	-
$Ni + L = NiL$	17.1	17.91	13.50	14.57	18.52	19.59	20.17	21.51	20.2	21.27
$Ni + H + L = NiHL$	20.14^+	21.12	18.60*	19.99	21.72	23.11	25.84	27.62	23.18^+	24.57
$2Ni + L = Ni_2L$	-	-	18.40*	20.01	-	-	25.76	27.91	-	-
$Al + L = AlL$	14.40	15.60	14.49^+	16.10	16.5	18.11	18.7	20.70	19.6	21.21
$Al + H + L = AlHL$	16.56^+	17.81	18.52^+	20.30	19.0	20.78	23.0	25.32	21.89^+	23.67
$Al + L = AlOHL + H$	9.45^+	10.48	9.19^+	10.48	10.67	11.96	11.3	12.86	11.68^+	12.97

* Stability constant for a contributing reaction (e.g., $ML + H = MHL$) was obtained at 20°C.

$^+$ Stability constant for a contributing reaction (e.g., $ML + H = MHL$) was determined at $\mu = 0.2$ and then corrected with Davies' equation to $\mu = .1$ to yield the value shown.

Table 9.2. Solid phases used to control metal ion solubility in soils.

	Reaction			log K°	log $K_{0.01}$
1.	$CaCO_3$(calcite) + $2H^+$	=	Ca^{2+} + CO_2(g) + H_2O	9.79	9.97
2.	Ca(soil)	=	Ca^{2+}	-2.50	-2.32
3.	$Ca_4H(PO_4)_3$ $5H_2O$(octacalcium phosphate)	=	$4Ca^{2+}$ + H^+ + $3PO_4^{3-}$ + $5/2H_2O$	-46.90	-44.96
4.	$Al(OH)_3$(gibbsite) + $3H^+$	=	Al^{3+} + $3H_2O$	8.04	8.45
5.	$AlPO_4$ $2H_2O$(varascite) + H_2O	=	$Al(OH)_3$(gibbsite) + PO_4^{3-} + $3H^+$	-30.12	-29.71
6.	$Fe(OH)_3$(soil) + $3H^+$	=	Fe^{3+} + $3H_2O$	2.70	3.10
7.	$Fe(OH)_3$(soil) + $3H^+$ + e^-	=	Fe^{2+} + $3H_2O$	15.73	15.91
8.	Fe^{3+} + e^-	=	Fe^{2+}	13.04	12.81
9.	Fe_3O_4(magnetite) + $8H^+$ + $2e^-$	=	$3Fe^{2+}$ + $4H_2O$	35.53	36.07
10.	$FeCO_3$(siderite) + $2H^+$	=	Fe^{2+} + CO_2(g) + H_2O	7.92	8.10
11.	FeS_2(pyrite) + $8H_2O$	=	Fe^{2+} + $2SO_4^{2-}$ + $14e^-$ + $16H^+$	-83.99	-83.45
12.	$Fe_3(PO_4)_2$ $8H_2O$(vivianite) + $2Al(OH)_3$(gibbsite) + $6H^+$ =		$3Fe^{2+}$ + $2AlPO_4$ $2H_2O$(variscite) + $10H_2O$	24.23	24.77
13.	$3Fe_3(PO_4)_2$ $8H_2O$(vivianite) + $2H^+$ + 8Ca(soil) =		$9Fe^{2+}$ + $2Ca_4H(PO_4)_3$ $5/2H_2O$(ocp) + $19H_2O$	-34.20	-32.57

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Table 9.2. Continued.

	Reaction	log K°	log $K_{0.01}$
14.	$3Fe_3(PO_4)_2\ 8H_2O$(vivanite) + $18H^+$ + $8CaCO_3$(calcite) = $9Fe^{2+}$ + $8CO_2(g)$ + $2Ca_4H(PO_4)_3\ 5/2H_2O$(ocp) + $27H_2O$	64.14	65.77
15.	MnO_2(pyrolusite) + $4H^+$ + $2e^-$ = Mn^{2+} + $2H_2O$	41.92	42.10
16.	γ-MnOOH(manganite) + $3H^+$ + e^- = Mn^{2+} + $2H_2O$	25.27	25.45
17.	$MnCO_3$(rhodochrosite) + $2H^+$ = Mn^{2+} + $CO_2(g)$ + H_2O	8.06	8.24
18.	MnS(alabanite) + $4H_2O$ = Mn^{2+} + SO_4^{2-} + $8e^-$ + $8H^+$	-33.59	-33.23
19.	Zn(soil) + $2H^+$ = Zn^{2+}	6.00	6.18
20.	α-ZnS(sphalerite) + $4H_2O$ = Zn^{2+} + SO_4^{2-} + $8e^-$ + $8H^+$	-44.53	-44.17
21.	Cu(soil) + $2H^+$ = Cu^{2+}	3.20	3.38
22.	Cu_2S(chalcocite) + $4H_2O$ + $2H^+$ = $2Cu^{2+}$ + SO_4^{2-} + $10e^-$ + $10H^+$	-74.46	-73.92
23.	$CdCO_3$(octavite) + $2H^+$ = Cd^{2+} + $CO_2(g)$ + H_2O	6.17	6.35
24.	CdS(greennokite) + $4H_2O$ = Cd^{2+} + SO_4^{2-} + $8e^-$ + $8H^+$	-47.81	-47.45
25.	$PbSO_4$(anglesite) = Pb^{2+} + SO_4^{2-}	-7.79	-7.43
26.	$Pb_3(PO_4)_2(c)$ + $2Al(OH)_3$(gibbsite) + $6H^+$ = $2AlPO_4\ 2H_2O$(variscite) + $3Pb^{2+}$ + $2H_2O$	15.83	16.37

Table 9.2. Continued.

	Reaction	log K°	$\log K^{\text{m}}_{0.01}$
27.	$3Pb_3(PO_4)_2(c) + 18H^+ + 8CaCO_3(calcite) = 9Pb^{2+} + 8CO_2(c) + 2Ca_4H(PO_4)_3\ 5/2H_2P(ocp) + 3H_2O$	38.95	40.58
28.	$3Pb_3(PO_4)_2(c) + 2H^+ + 8Ca(soil) + 5H_2O = 9Pb^{2+} + 2Ca_4H(PO_4)_3\ 5/2H_2O(ocp)$	-59.40	-57.77
29.	$PbCO_3(cerussite) + 2H^+ = Pb^{2+} + CO_2(g) + H_2O$	4.65	4.83
30.	$PbS(galena) + 4H_2O = Pb^{2+} + SO_4^{2-} + 8e^- + 8H^+$	-48.23	-47.87
31.	$Ni_3(PO_4)_2(c) + 6H^+ + 2Al(OH)_3(gibbsite) = 3Ni^{3+} + 2AlPO_4\ 2H_2O(variscite) + 2H_2O$	29.82	30.36
32.	$3Ni_3(PO_4)_2(c) + 18H^+ + 8CaCO_3(calcite) = 9Ni^{2+} + 8CO_2(g) + 2Ca_4H(PO_4)_3\ 5/2H_2O(ocp) + 3H_2O$	78.39	80.01
33.	$3Ni_3(PO_4)_2(c) + 2H^+ + 8Ca(soil) + 5H_2O = 9Ni^{2+} + 2Ca_4H(PO_4)_3\ 5/2H_2O(ocp)$	-19.98	-18.36
34.	$NiCO_3(c) + 2H^+ = Ni^{2+} + CO_2(g) + H_2O$	7.45	7.63
35.	$NiS(c) + 4H_2O = Ni^{2+} + SO_4^{2-} + 8e^- + 8H^+$	-40.63	-40.27

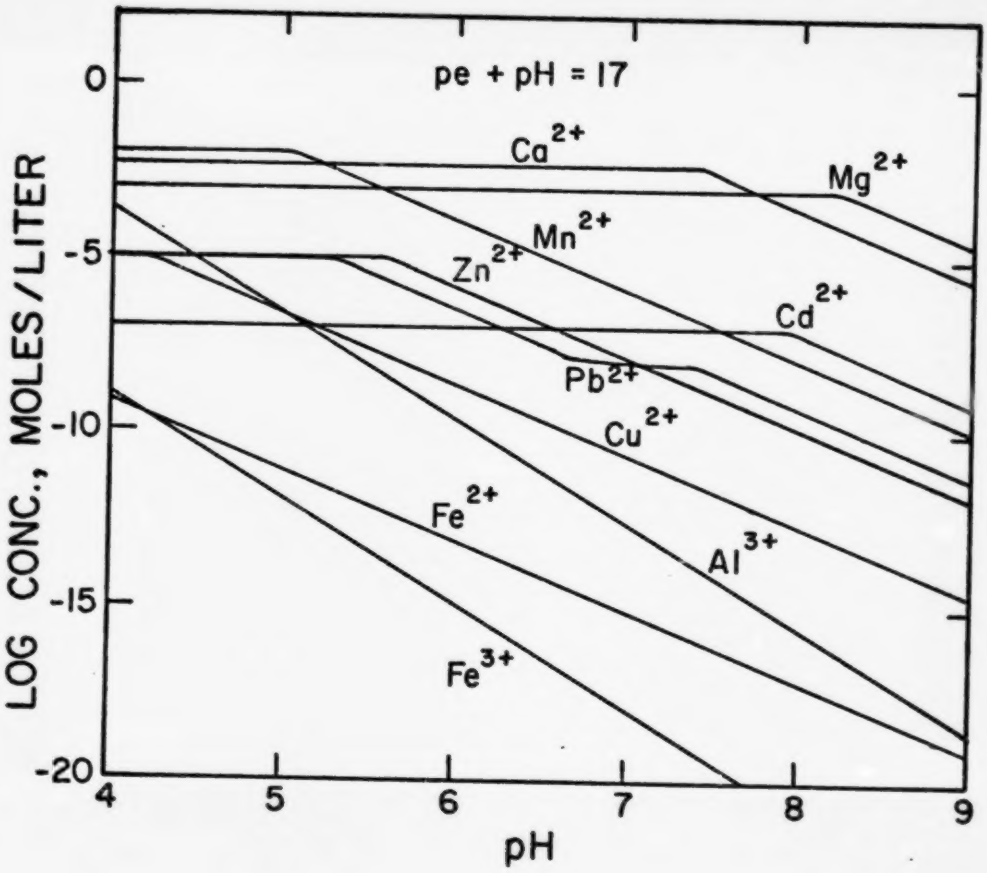

Figure 9.1. Solubility relationships for metals in soil at pe + pH of 17.

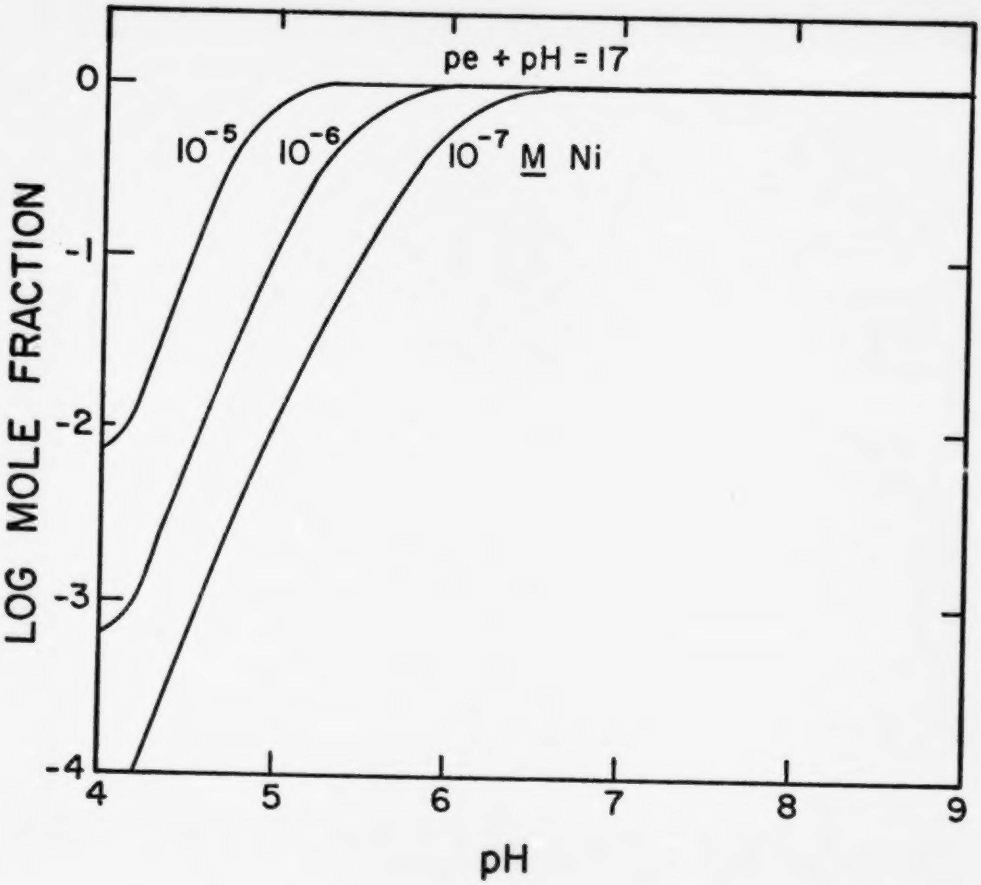

Figure 9.2. Effect of soluble levels of Ni on stability diagram for DTPA in soils at pe + pH of 17.

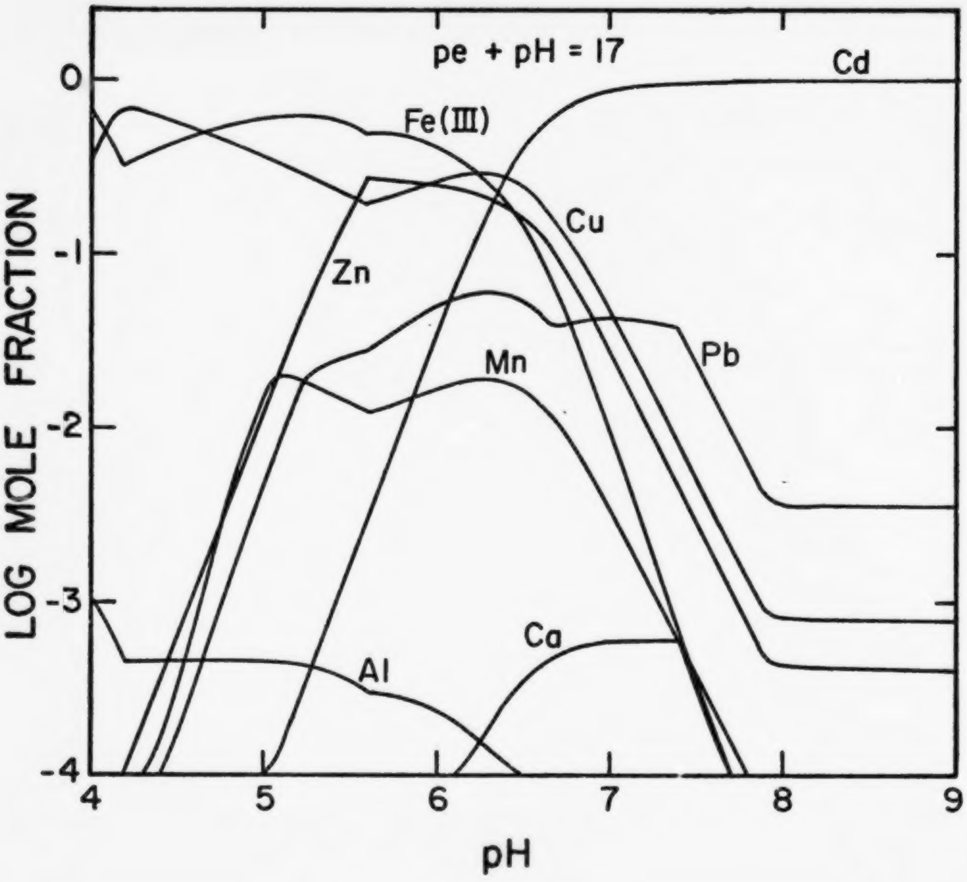

Figure 9.3. Stability diagram for DTPA in soils at pe + pH of 17.

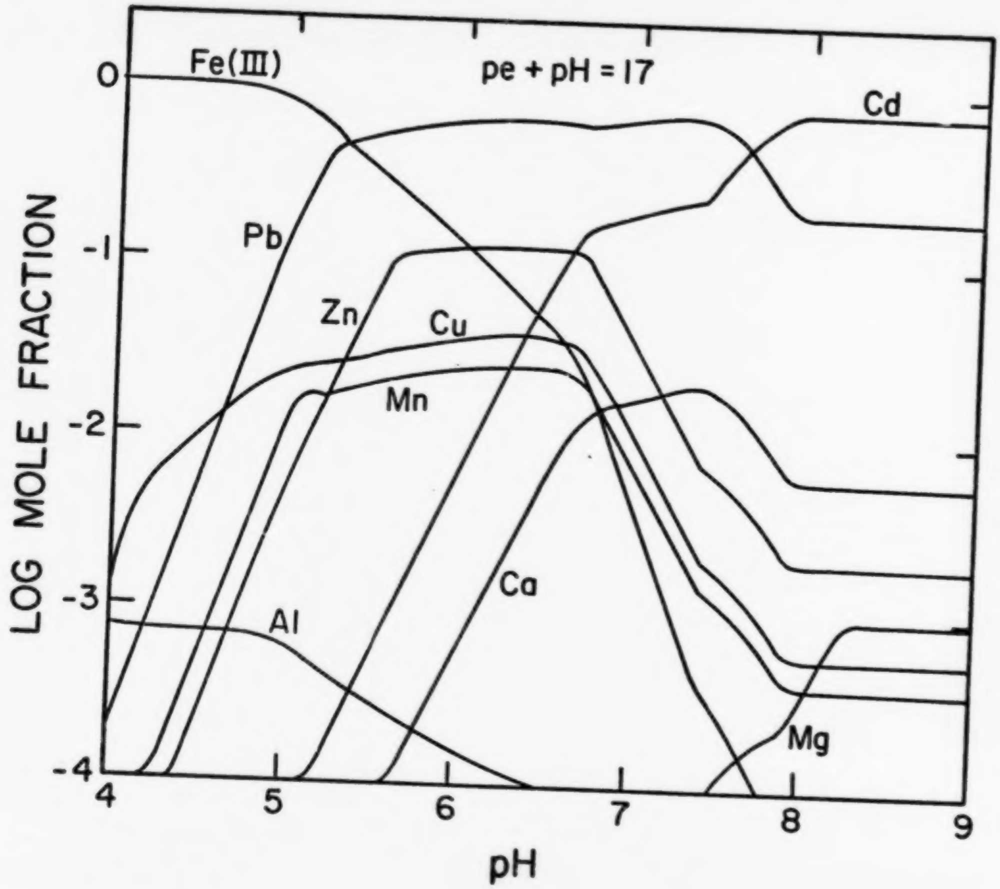

Figure 9.4. Stability diagram for EDTA in soils at pe + pH of 17.

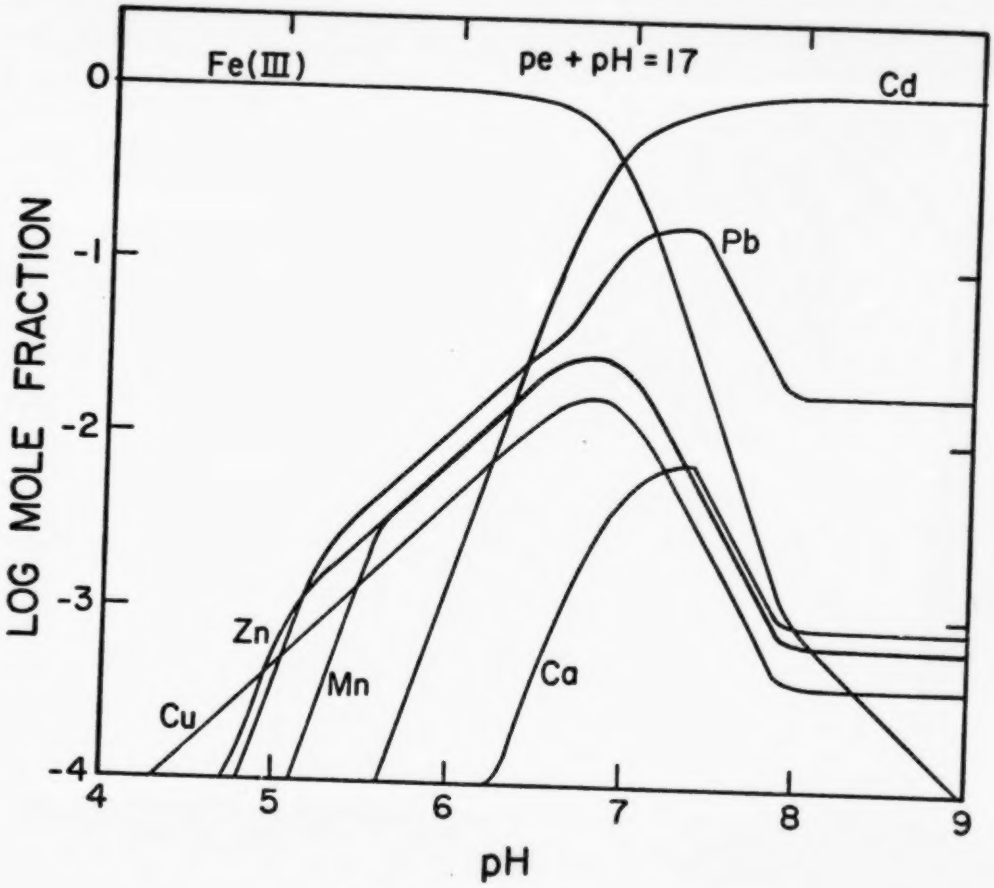

Figure 9.5. Stability diagram for CDTA in soils at pe + pH of 17.

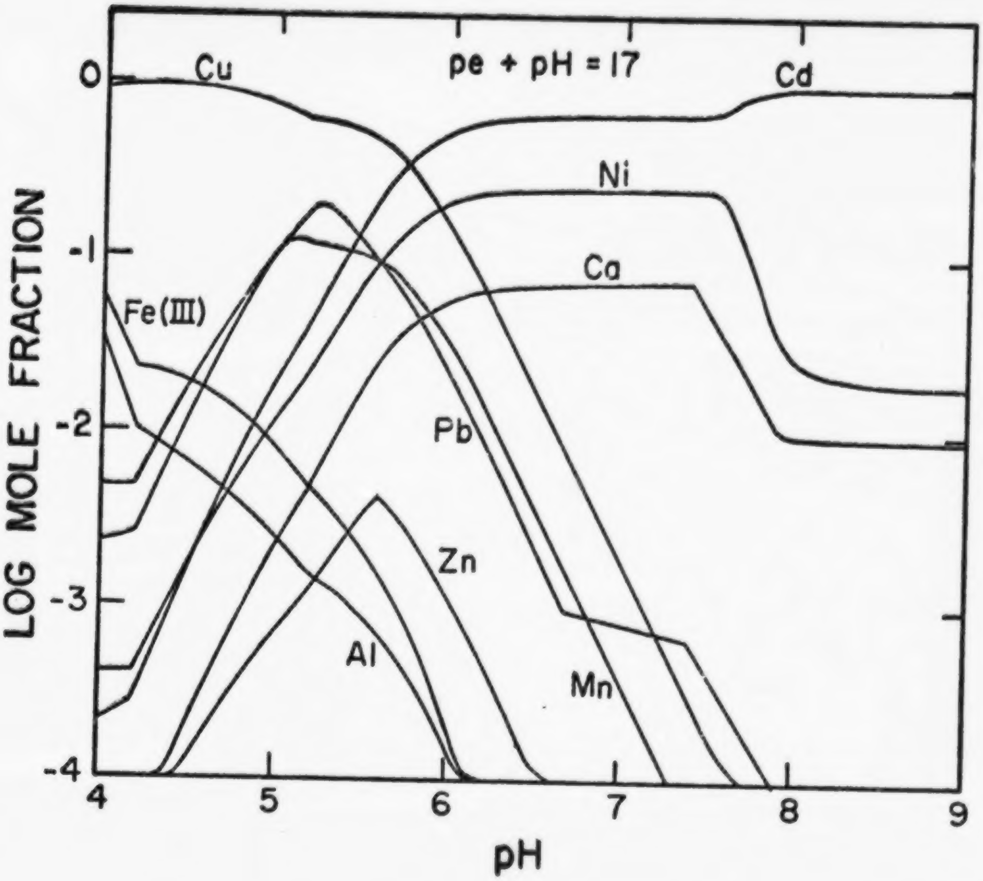

Figure 9.6. Stability diagram for EGTA in soils at pe + pH of 17.

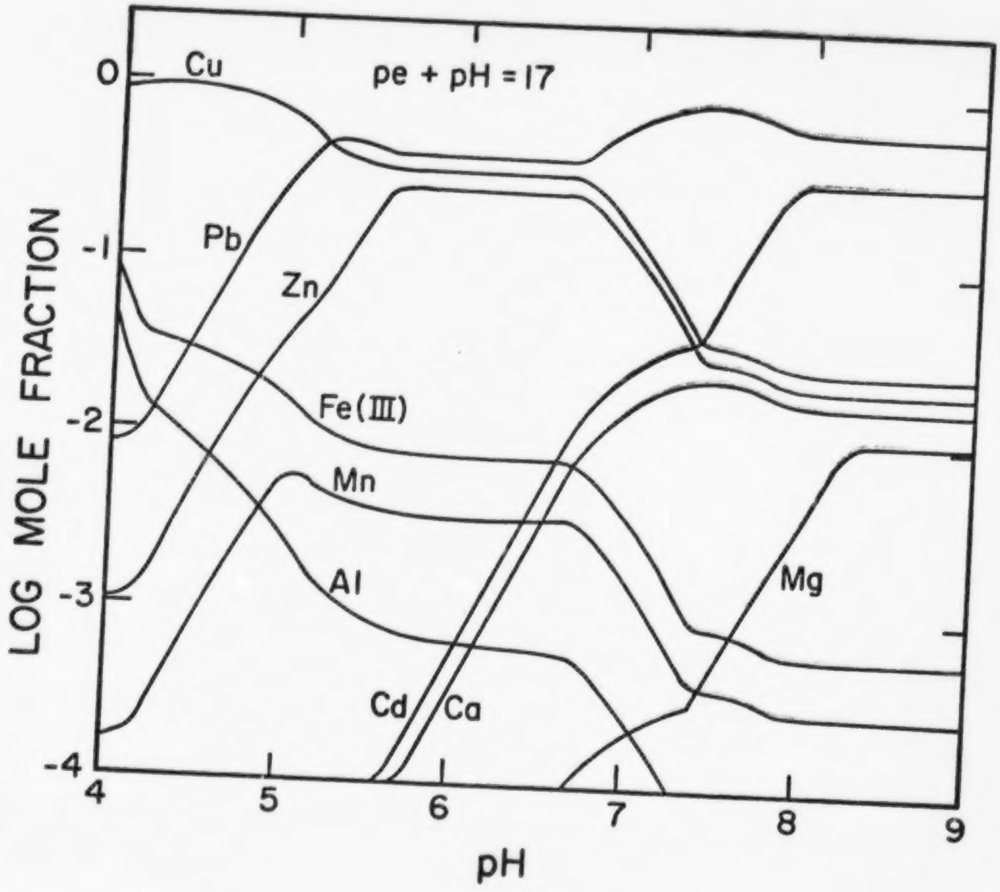

Figure 9.7. Stability diagram for HEDTA in soils at pe + pH of 17.

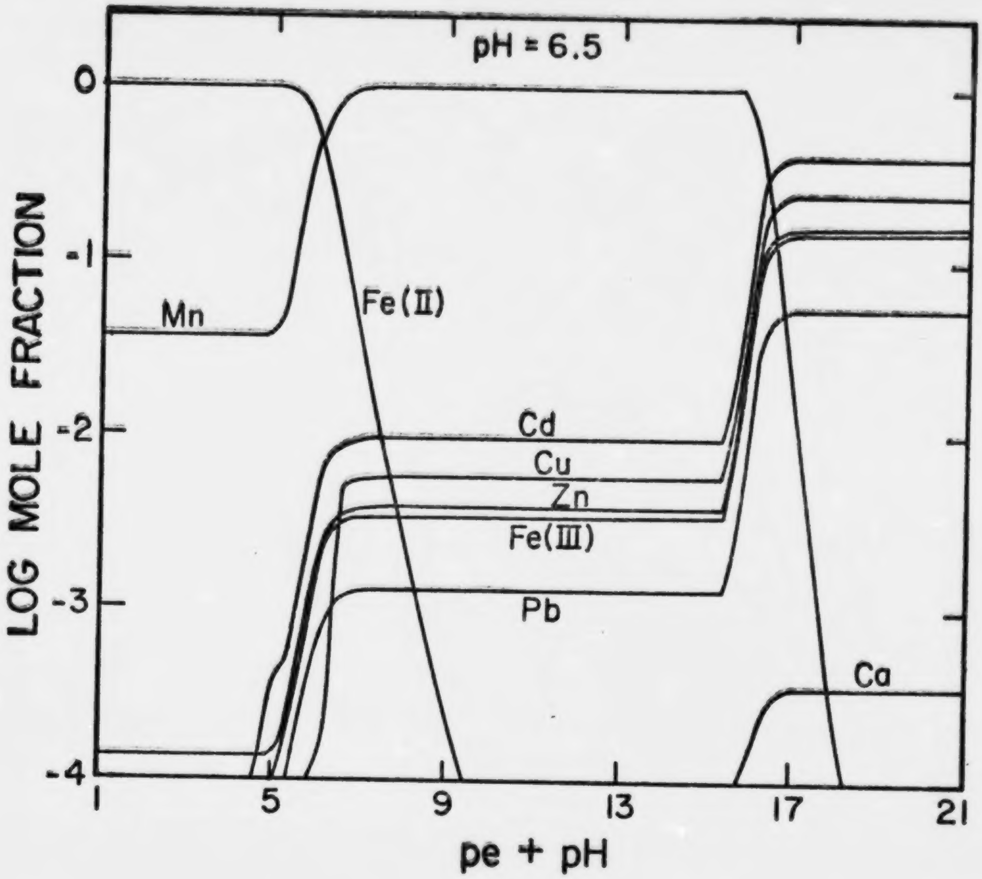

Figure 9.8. Stability diagram for DTPA in soils at pH 6.5 with precipitation of Cu_2S, PbS, and CdS.

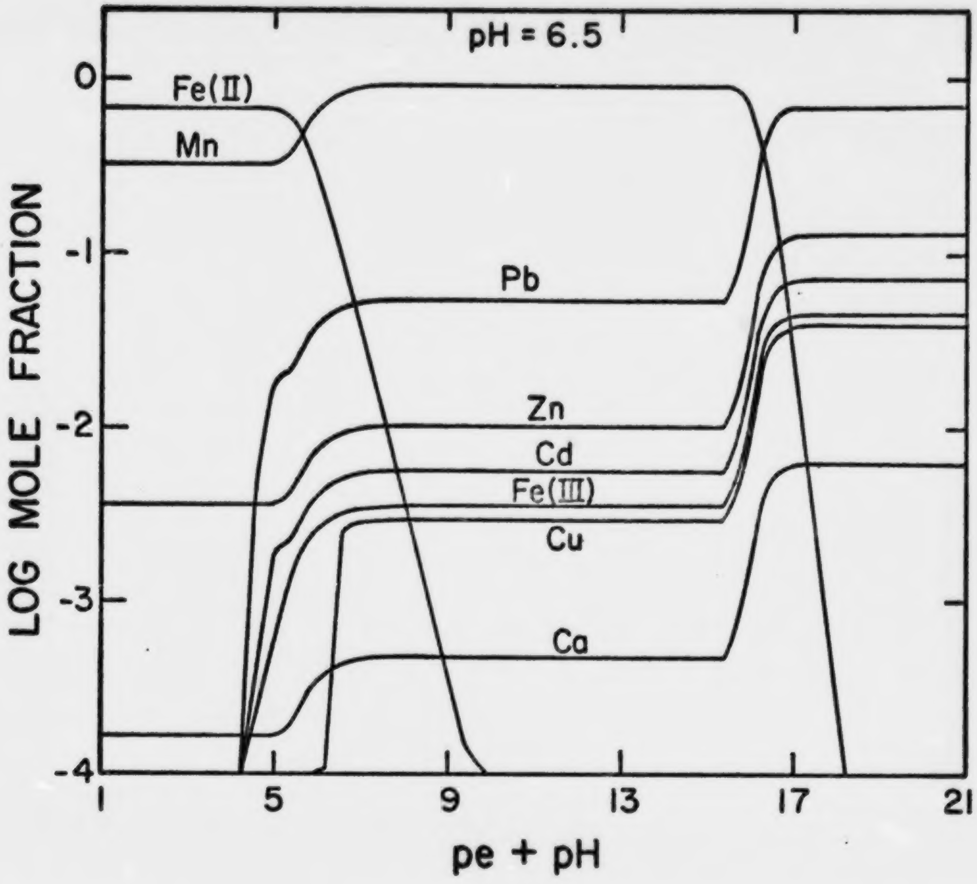

Figure 9.9. Stability diagram for EDTA in soils at pH 6.5 with precipitation of Cu_2S, PbS and CdS.

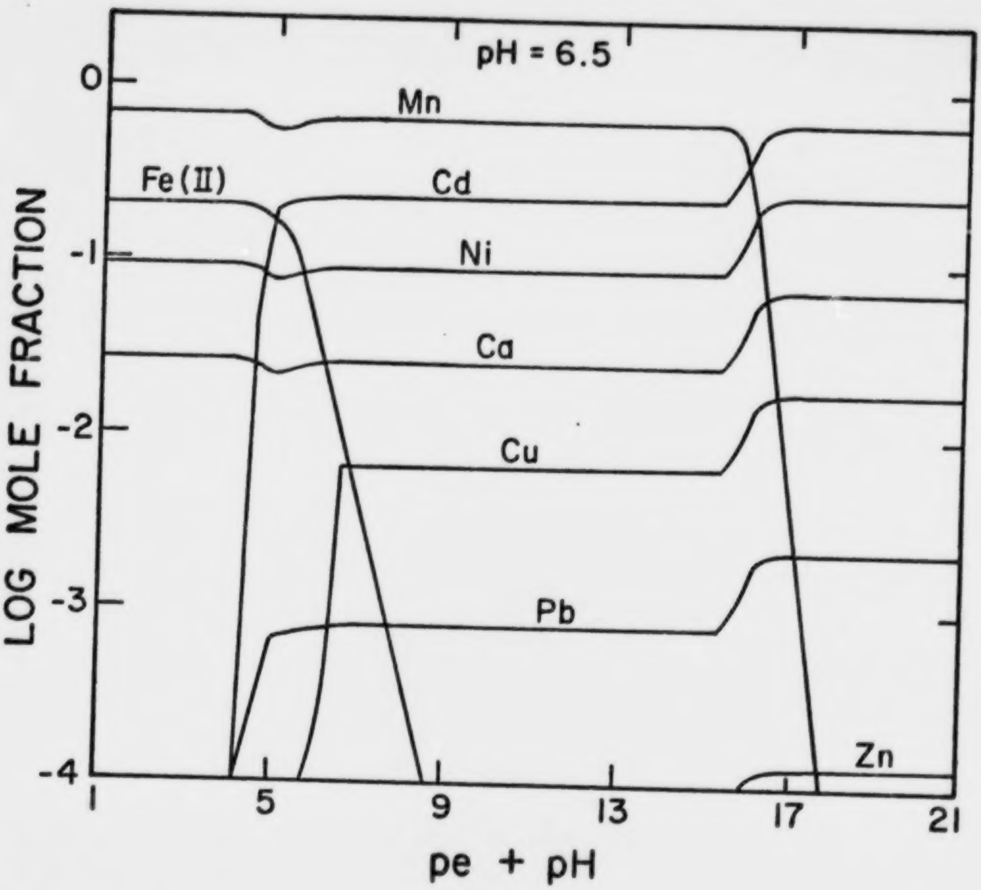

Figure 9.10. Stability diagram for EGTA in soils at pH 6.5 with precipitation of Cu_2S, PbS and CdS.

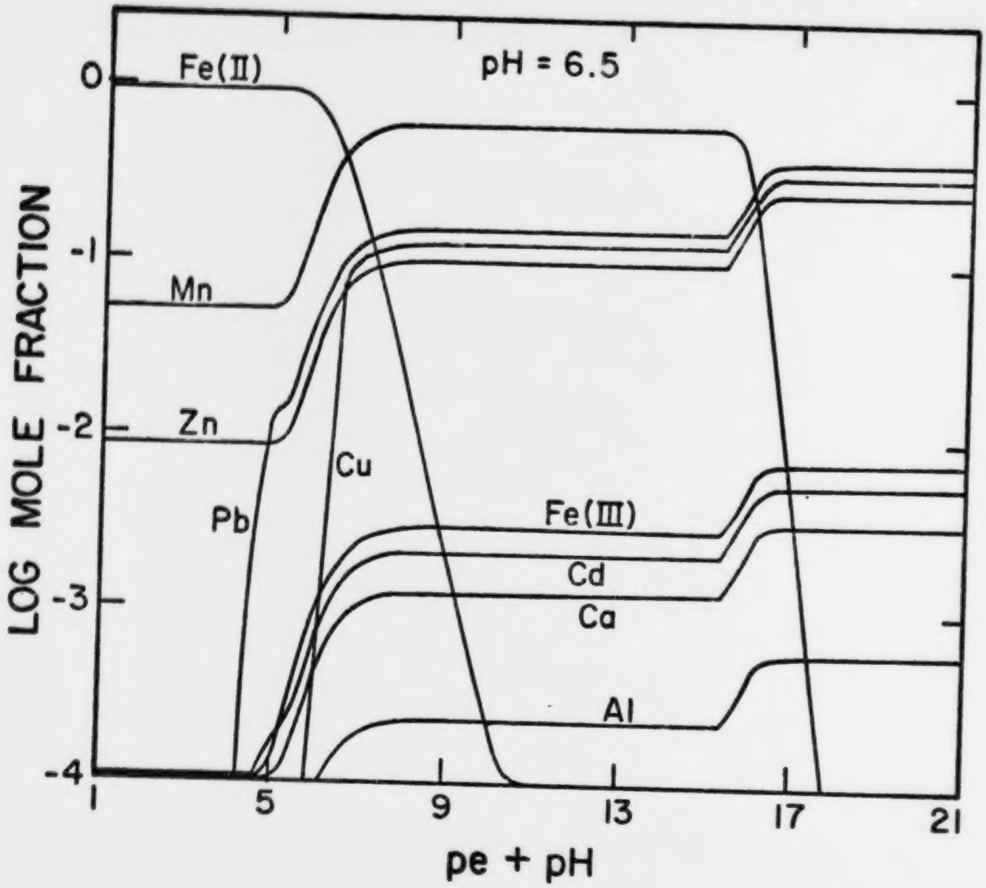

Figure 9.11. Stability diagram for HEDTA in soils at pH 6.5 with precipitation of Cu_2S, PbS and CdS.

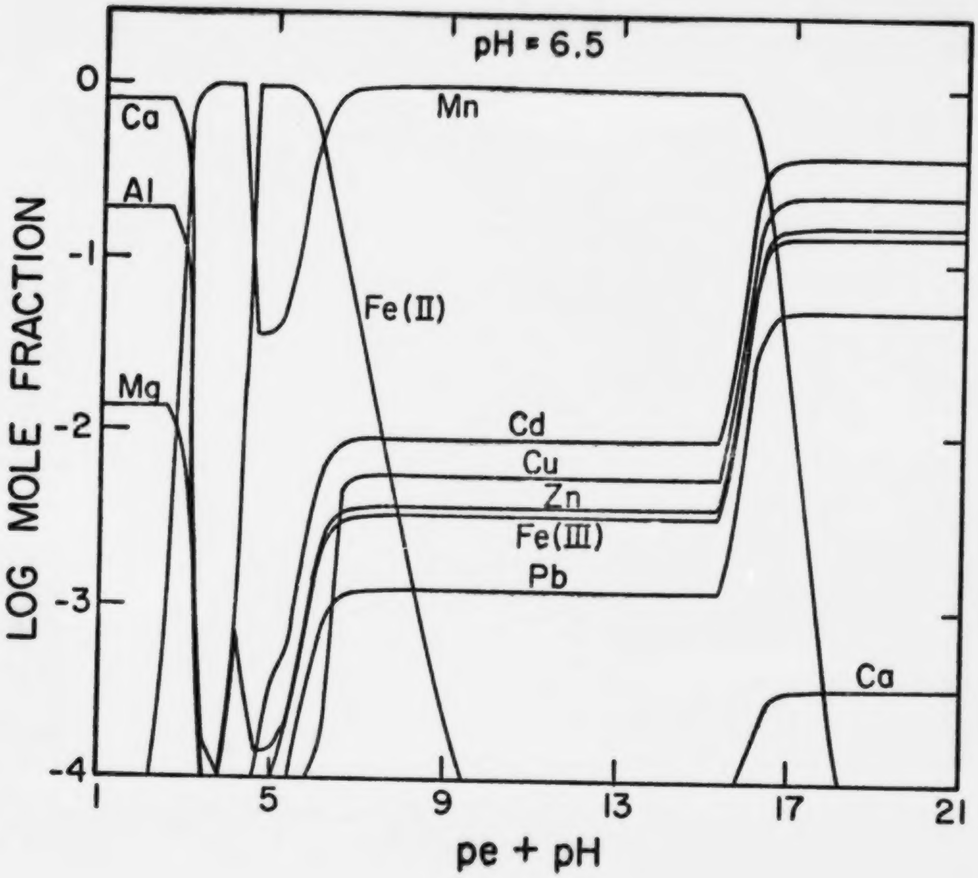

Figure 9.12. Stability diagram for DTPA in soils at pH 6.5 with precipitation of Cu_2S, PbS, ZnS, MnS, and FeS_2.

SECTION 10

TRANSFORMATIONS OF CARBON, NITROGEN AND METALS IN SLUDGE-AMENDED SOILS

INTRODUCTION

One of the potential problems associated with sewage sludge application on land is the elevated concentrations of heavy metals (Cu, Zn, Pb, Cd, and Ni) present in many sludges (Page, 1974; Sommers, 1977; C.A.S.T., 1976). Besides being phytotoxic and potentially detrimental to human and animal health, heavy metals can also decrease decomposition of organic matter in soils (Bhuiya and Cornfield, 1972; Ruhling and Tyler, 1973; Jordan and Lechevalier, 1975; Mathur et al., 1979). Since deomposition of sewage sludge in soils is an important step in the cycling of plant nutrients, and since the rates of sludge organic N mineralization control, in part, the annual amounts of sludge that can be applied to soils, knowledge of heavy metal effects on decomposition in sludge-soil systems is needed.

Field and laboratory research has shown that decomposition of sludge organic C under aerobic conditions results in CO_2 being the major end product. A laboratory study by Agbim et al. (1977) indicated that the percent of sludge organic C evolved as CO_2 from soils decreased with increasing rate of sludge application. Miller (1974) has reported that from 18 to 20% of sludge organic C is evolved as CO_2 in the first six months of a soil + sludge incubation. In the same study, soil texture and water content were not found to greatly influence sludge decomposition. Similarly, Sommers et al. (1976) conducted a one year sludge decomposition study and concluded that sludge composition rather than soil properties (i.e., pH, texture) determines the amount of sludge decomposition in soil. Analysis of organic C in samples from field plots has indicated that ~ 50% of sludge organic C was decomposed over a 6 year period (Varanka et al., 1976). Despite the data available on sludge decomposition, it is difficult to compare data and conclusions from different studies since sludge properties vary widely for different treatment plants (i.e., a function of treatment and handling processes) and they vary with time at a given sewage treatment plant. These variations in sludge composition cause problems when attempting to interpret decomposition data from the viewpoint of potential heavy metal effects. One approach used to overcome the problem of variability in sludge composition is the preparation of synthetic sludges from known materials (Terry, 1976; Silviera, 1976). These authors prepared a synthetic sludge by anaerobically digesting dried yeast cells in a nutrient solution containing different amounts of heavy metal salts to produce a sludge that varied only in heavy metal concentrations.

Preliminary soil incubation studies indicated that heavy metal levels in the sludge had little effect on sludge decomposition rates.

Mineralization of organic N involves the conversion of organic N to inorganic N, e.g., decomposition of amino acids to yield NH_4^+. The rate of mineralization of sludge organic N depends on soil environmental factors, as well as the properties and rate of application of the sludge. A 16 week laboratory incubation study has shown that mineralization of sludge organic N ranged from 4 to 48% of added organic N (Ryan et al., 1973). Utilizing laboratory soil cylinders, King (1973) reported approximately 60% mineralization of sludge organic N within 18 weeks and noted that incorporated and surface-applied sludge treatments mineralized organic N at similar rates. Laboratory studies have shown that sludge organic N mineralization is affected by sludge treatment processes (Epstein et al., 1978; Chromec and Magdoff, 1977). Results from a field study indicate the percent mineralization of sludge organic N decreases with time; the percent of sludge organic N mineralized being 55, 25 to 30, and 10 to 15% for the first, second, and third cropping seasons, respectively (Kelling, 1978).

It is often difficult to develop and interpret mineralization data from sludge incubation studies conducted in the laboratory because of the wide variation in sludge properties and nitrogen losses resulting from NH_3 volatilization and denitrification. To counter some of these problems, Terry (1976) produced ^{15}N labelled synthetic sludge from yeast cells. Results from subsequent soil-sludge incubation studies showed that immobilization and mineralization occur simultaneously with mineralization reaching 29 to 40% of added sludge organic N in 6 months. It was also noted in this study that immobilization and mineralization of N were not greatly influenced by soil texture, moisture, or pH, but mineralization of organic N increased with increasing incubation temperature. Overall sludge C/N ratios, which are generally less than 15/1, would suggest that mineralization would be prevalent following sludge application to soil; however, sludges contain appreciable amounts of nonaqueous solvent extractable compounds (e.g., fats and oils) that are devoid of N and will result in N immobilization upon decomposition. In addition to the above factors that affect N mineralization in sludge amended soils, sludges contain varying amounts of trace elements (i.e., Zn, Cu, As, Cd) which have been shown to either increase or decrease N mineralization (Quraishi and Cornfield, 1971; Premi and Cornfield, 1969; Premi, 1970; Liang and Tabatabai, 1977).

Nitrification of NH_4^+-N in sludge amended soils has been studied. Several studies have shown that nitrification can be partially inhibited by sludge applied at rates of 50 metric tons/ha (Premi and Cornfield, 1969), 36 metric tons/ha (Wilson, 1977a), and 18 metric tons/ha (Ryan et al., 1973). One explanation proposed for decreased nitrification rates at elevated sludge application rates is the presence of toxic concentrations of heavy metals in the sludges. Nitrification inhibition in soils amended with heavy metal salts has been reported (Premi and Cornfield, 1969; Wilson, 1977b; Liang and Tabatabai, 1978). In addition, Wilson (1977a) reported that the addition of salts of Cd, Zn, or Cu to sewage sludge delayed nitrification in sludge amended soils. However, most studies of heavy metal effects on nitrification have used heavy metal salts which are usually more biologically available than the indigenous metals in sludges (CAST, 1976).

Studies with synthetic anaerobically digested sludges prepared from yeast cells and containing varying amounts of heavy metals (Cd, Zn, Cu, Pb, and Ni) indicated that sludges containing high amounts of heavy metals result in a temporary inhibition of nitrification. Recent research on intact soil cores amended with sludge indicates that leaching of NO_3^- is minimal in soils receiving sludges (Sommers et al., 1979). Extensive losses of N added to soils in sewage sludges has been reported in laboratory (King, 1973) and field studies (Kelling et al., 1977). Denitrification in anaerobic microsites and/or immobilization can provide pathways for NO_3^- losses from sludge amended soils.

Limited research has been done to determine metal forms present in sludge or sludge amended soil such as metal precipitates, the stability organo-metallic complexes, and sorption products (Stover et al., 1976; Bloomfield and Pruden, 1975; Tan et al., 1971; Holtzclaw et al., 1978; Baham et al., 1978; Boyd et al., 1979). It is also of interest to develop an extractant that would predict the plant availability of heavy metals in the soils. Symeonides and McRae (1978) have assessed plant available Cd in soils treated with $CdCl_2$ and found that a 1N NH_4NO_3 extractant was a good predicter of Cd available to radishes. The availability of heavy metals in sludge treated soils has been assessed by a DTPA (diethylenetriamine pentaacetic acid) extractant which had been developed as a soil test for Fe, Mn, Zn and Cu in calcareous soils (Lindsay and Norvell, 1978). Significant correlations have been reported between DTPA extractable metals in sludge amended soils and metals taken up by corn (Street et al., 1978; Kelling et al., 1977), rye and sorghum (Kelling et al., 1977), wheat (Sims and Boswell, 1978; Mitchell et al., 1978), lettuce (Mitchell et al., 1978) and snap beans (Latterell et al., 1978). Following addition of sludge to soil, heavy metals generally become less available to plants with time (Kelling et al., 1977; Chaney, 1973). However, DTPA extractable metals can also remain constant or increase with time after amending soils with sludge (Gaynor and Halstead, 1976; Silviera and Sommers, 1977). The changes in metal extractability with time may result from various transformations (i.e., release of metals from decomposing organic matter, oxidation of S in metal sulfides, precipitation or dissolution of metal solid phases).

Significant correlations between DTPA extractable metals and the amount of metals added to the soil in sludge have been reported (Bingham et al., 1975; Sommers et al., 1976). However generalizations concerning DTPA extractable metal values to all sludge amended soils is generally not possible since metals do not exist in the same form in different sludges (Stover et al., 1976). Therefore, DTPA extractable metals in soils amended with different sludges can be variable (Silviera and Sommers, 1977). It is this variability in sludge properties that precludes generalizations concerning the fate of metals in sludge-amended soils.

To obviate the problems of variability in sludge properties, Silviera (1976) produced anaerobically digested synthetic sludges from dried yeast cells, inorganic nutrients, and heavy metal salts. The digested synthetic sludges were uniform in properties except for varying concentrations of heavy metals. Following application of the synthetic and several municipal sludges to soil, values from DTPA extractions of the soil indicated that the synthetic sludge metals behaved similarly to those from municipal sludges.

The objectives of this study were to evaluate the effects of metals on the decomposition of organic C, and mineralization of organic N in sludge-amended soils.

MATERIALS AND METHODS

Nine anaerobically digested synthetic sludges and three municipal sludges were used in this study. Synthetic sludges were prepared by mixing 100 g of dried yeast cells (*Candida utilus*), 10 g $CaCO_3$, 10 g KH_2PO_4, 10 g $FeSO_4 \cdot 7H_2O$, 5 g $MgCl_2 \cdot 6H_2O$, 1.600 ml H_2O, and varying concentrations of heavy metal salts in 2 liter Erlenmeyer flasks. The flasks were stoppered and placed on magnetic stirrers which maintained a temperature of 35C. The mixtures were inoculated with 5 ml of active anaerobic sludge from the W. Lafayette, IN, treatment plant, and adjusted periodically to pH 7 with solid potassium hydroxide pellets until the onset of methane generation. Gas samples obtained from the head space were analyzed by gas chromatography to confirm methane production during digestion. The following amounts of metals were added to the flasks prior to digestion: 96.1 mg $Cu(NO_3)_2 \cdot 3H_2O$, 227.2 mg $Zn(NO_3)_2 \cdot 6H_2O$, 13.7 mg $Cd(NO_3)_2 \cdot 4H_2O$, 40 mg $Pb(NO_3)_2$, 123.7 mg $Ni(NO_3)_2 \cdot 6H_2O$. Other digesters contained 0, 2, 4, and 8 times these amounts. The sludges are referred to as S0, S1, S2, S3, and S4 to indicate the relative heavy metal concentrations. The reader is referred to Sommers *et al*. (1976) for further details of synthetic sludge preparation. The properties of the synthetic sludges prepared are listed in Table 10.1.

The three municipal sludges were obtained from drying beds at the Anderson, Marion, and Frankfort, Indiana sewage treatment plants. Properties of the municipal sludges are listed in Table 10.1. Synthetic and municipal sludges were air-dried and ground to pass 60 mesh. The soil used in this study was a surface sample (0-15 cm) of a Chalmers silt loam (Typic Argiaquoll).

The analytical methods for soil and sludge analysis were as follows: total solids and volatile solids were determined gravimetrically after drying at 105 C (16 hrs.) and igniting at 600C (6 hrs.), total C was determined by a modified Allison (1960) technique, whereby the CO_2 liberated by H_2SO_4-H_3PO_4-$K_2Cr_2O_7$ digestion was trapped in NaOH. The NaOH trapping solution was treated with excess 1.5 *M* $BaCl_2$, and titrated with standard HCl; inorganic C was similarly determined by digestion of samples with dilute H_2SO_4 as an antioxidant (Allison, 1960); organic C was obtained by difference. Soluble and exchangeable NH_4^+ and $NO_2^- + NO_3^-$ were determined by steam distillation technique after 2 *M* KCl addition (Bremner, 1965). Samples digested with HNO_3-$HClO_4$ (Sommers and Nelson, 1972) were analyzed for total Cu, Zn, Cd, Pb, and Ni on a Varian AA-6 atomic absorption spectrophotometer using a hydrogen continuum background corrector. Metals extractable with DTPA (Cu, Zn, Cd, Pb, and Ni) were determined by shaking (2 hrs) soil samples (1 g) with 10 ml of a solution (pH 7.3) containing 0.005 *M* DTPA, 0.01 *M* $CaCl_2$ and 0.1 *M* triethanolamine (Lindsay and Norvell, 1978) followed by filtration and atomic absorption spectrophotometry.

The sludge incubation study involved amending 100 g of Chalmers soil with 1, 2, 3, and 4 g (i.e., 22, 45, 67, and 90 metric tons/ha) of air-dried synthetic or municipal sludge. The soil-sludge mixtures were placed in one liter Erlenmeyer flasks and the moisture adjusted to approximately -0.6 bar tension (0.211 g H_2O/g soil) with an $(NH_4)_2SO_4$ solution. The amount of $(NH_4)_2SO_4$ added adjusted the inorganic N content of all sludge amended soils to a constant level (25 mg N/g sludge added). Sludge-amended soils were also amended with $CaCO_3$ to adjust all treatments to the inorganic C level of the 2 g Marion sludge/100 g soil (i.e., 9.5 mg $CaCO_3$/g soil). Non-sludge treated controls included were (per 100 g soil): 1) no amendment; 2) 0.949 g $CaCO_3$; 3) 9.949 g $CaCO_3$ and 0.25 mg NH_4^+-N; and 4) 0.949 g $CaCO_3$ and 100 mg NH_4^+-N. The soil-sludge mixtures were aerated with CO_2-free moist air at a rate of 8 ml/min in a closed constant temperature (23 C) system (Terry, 1976). Carbon dioxide evolved was collected in 25 ml of 1 M KOH and determined periodically by adding excess 1.5 M $BaCl_2$ to 10 ml portions of the KOH and titrating residual alkalinity with standard HCl using phenolphthalein as an indicator. All incubations were conducted in duplicate for 240 days.

Since all sludges studied contained $CaCO_3$, a study was performed to evaluate the contribution of $CaCO_3$ dissolution to the total CO_2 evolved from sludge-treated soils. Carbon 14 labelled $Ca^{14}CO_3$ (relative specific activity 5.23×10^5 cpm/g) was prepared by treating a $Na^{14}CO_3$ solution (ICN Chemical and Radioisotope Division) with $Ca(OH)_2$ to precipitate $Ca^{14}CO_3$. The $Ca^{14}CO_3$ was washed with distilled water and dried at 105 C.

The apparatus used in the soil-sludge-$CaCO_3$ study was identical to that previously described. In this study 100 g of Chalmers soil were amended with one and four grams (i.e., 22 and 90 metric tons/ha) of municipal sludge (Anderson, Marion, and Frankfort). The sludge-amended soils were also amended with $Ca^{14}CO_3$ to adjust all treatments to 9.5 mg $CaCO_3$/g soil. The Marion sludge treatment received no $Ca^{14}CO_3$. The soil-sludge-$CaCO_3$ mixtures were placed in one liter Erlenmeyer flasks and the moisture was adjusted to -0.6 bar tension with an $(NH_4)_2SO_4$ solution. The amount of $(NH_4)_2SO_4$ added adjusted the inorganic N content of all sludge amended soils to a constant level (25 mg N/g sludge added). Control treatments included were (per 100 g soil): 1) no amendment; 2) 0.949 g $Ca^{14}CO_3$; 3) 0.949 g $Ca^{14}CO_3$ and 25 mg NH_4^+-N; and 4) 0.949 g $Ca^{14}CO_3$ and 100 mg NH_4^+-N. The incubation was conducted in duplicate for 150 days. The evolution of $^{14}CO_2$ was measured by removing a 2 ml aliquot of the KOH solution, acidifying with 2 N HCl and collecting the CO_2 released in 2.5 ml of NCS reagent (quarternary ammonium base in toluene, Amersham/Searle Corp.) in a scintillation vial fitted with a glass bead absorption tower. After trapping the CO_2, the tower contents were washed into the scintillation vial with PPO-toluene cocktail (0.5% w/w 2,5-diphenyl oxazole in toluene) and the ^{14}C activity was measured on a Packard 2420 scintillation counter. Counting efficiency was corrected by assaying ^{14}C-toluene standards in a NCS matrix. Residual $CaCO_3$ was also measured in selected soils by the procedure of Bundy and Bremner (1972).

To further evaluate mineralization and nitrification in sludge-amended soils as related to soil pH and methods of sludge handling, a second soil-sludge incubation was performed, whereby liquid and dried municipal sludge were incubated with the Chalmers soil. Liquid sludge was obtained directly from the anaerobic digestor at the West Lafayette, Indiana, sewage treatment plant. The liquid sludge was stored at 4°C while a subsample was placed on a laboratory scale sand drying bed (32 x 28 cm with 6 cm of quartz sand above gravel) and allowed to dry for three weeks. The dried sludge was collected and ground to < 50 mesh. The sludge supernatant that leached through the sand and gravel, was collected from a drainage tube at the bottom of the sand drying bed. Properties of the liquid and dried sludges are shown in Table 10.1.

The design of the experiment involved mixing liquid and dried sludge with 100 g of Chalmers soil at rates equivalent to 15, 60, and 100 metric tons/ha (i.e., 0.66, 2.64 and 4.40 g/100 g soil). Soil moisture was adjusted to -0.6 bar tension and $(NH_4)_2SO_4$ solution was added to adjust all treatments to 1.648% NH_4^+-N on a sludge oven-dry weight basis. On a soil basis, the concentrations of NH_4^+-N added were 110, 430, and 704 µg N/g soil for the 15, 60, and 100 metric tons/ha sludge addition rates, respectively. In order to maintain soil moisture at -0.6 bar for the 60 and 100 metric tons/ha rates, the liquid sludge was centrifuged at 1,000 x g to increase the solids content with the supernatant being discarded. The NH_4^+-N added was adjusted to levels shown above by adding an appropriate amount of $(NH_4)_2SO_4$ solution. The supernatant collected from the drying bed was added to one treatment in an amount that supplied the inorganic N present in the 15 metric tons/ha rate (110 µg N/g). Duplicate treatments of the soil-sludge mixtures were placed in one liter flasks and intimately mixed. The flasks were covered with parafilm and incubated in a constant temperature (23°C) room for 66 days. Five gram soil samples were removed periodically, extracted with 2 M KCl extraction, and analyzed for NH_4^+ and NO_2^-+NO_3^- by steam distillation (Bremner, 1965). Soil pH was measured in a 2:1 water:soil ratio on a Sargent Welch pH meter fitted with a combination glass electrode.

All data presented are averages of duplicate incubation systems. Variations between duplicates was generally less than 10%.

RESULTS AND DISCUSSION

$CaCO_3$ Dissolution in Sludge-Amended Soils

Almost all sewage sludges contain substantial amounts of carbonates that are present either as a result of lime addition to facilitate dewatering, as a component of the influent sewage, or as a result of precipitation during anaerobic digestion. Preliminary data on sludge material taken from various treatment plants throughout the U.S.A. showed that the inorganic C content ranged from 0.06 to 3.4% of the sludge dry weight corresponding to 0.5 to 28.33% as $CaCO_3$ (unpublished data). The Marion sludge used in this study contained 5.57% inorganic C (46.4% as $CaCO_3$). Presumably, the majority of the inorganic C analyzed was due to $CaCO_3$, however, other Ca or Mg carbonates may be present (e.g., dolomite, Silviera *et al*., 1977). The presence of $CaCO_3$ in sludges can influence results obtained from decomposition studies in two ways: 1) CO_2 originating from dissolution of $CaCO_3$ and from decomposition of organic C must be differentiated, and 2) decomposition may increase due to an increase in soil pH. Therefore, it was necessary to determine the contribution of $CaCO_3$ dissolution to CO_2 evolution from soil and sludge amended soils. Figure

10.1 shows that the addition of $CaCO_3$ to Chalmers soil almost doubled CO_2 production during the first 10 days of incubation and that addition of $CaCO_3$ and $(NH_4)_2SO_4$ (250 µg and 1000 µg N/g) further increased CO_2 evolution. Analysis of carbonate in the soils at the end of the study showed that from 66 to 98% of the initial $CaCO_3$ added to the soil was dissolved (Table 10.2), indicating that $CaCO_3$ dissolution contributed significantly to the total CO_2 evolved. To differentiate between the CO_2 resulting from organic C decomposition and that from $CaCO_3$ dissolution, $Ca^{14}CO_3$ was prepared and added to soils at the same rates as used in experiments with sludge additions. The data obtained (Table 10.3) indicate that most of the increase in CO_2 evolution in the $CaCO_3$ amended soils was due to $CaCO_3$ dissolution; however, enhanced decomposition of soil organic matter also resulted from the increased soil pH. From 52 to 84% of added $Ca^{14}CO_3$-C was evolved as $^{14}CO_2$ in the Chalmers soil within 151 days. Most of the $CaCO_3$ dissolution occurred within the first 18 days with 30-55% of the $CaCO_3$-C evolved during the first 3 days of the incubation (Figues 10.2 and 10.3). Microbial decomposition of soil or sludge organic C is likely minimal during this 3 day time period. Varying percentages of $CaCO_3$ dissolution in the Chalmers soil treatments were caused by differences in soil pH elicited by H^+ production during nitrification. As shown in Table 10.2, the pH of soils receiving 0, 250, and 1000 µg N/g were 8.0, 7.6, and 6.2 after 151 days of incubation. When CO_2 evolved from the Chalmers soils amended with N and $CaCO_3$ was corrected for C originating from $CaCO_3$, the values for CO_2-C evolved are still as much as 31% greater than that of the soil not treated with N or $CaCO_3$. This increase in CO_2 production was apparently due to increased microbial activity and decomposition of soil organic matter at pH > 7.0. Tester et al. (1979) reported the decomposition of soil organic C was increased by 82% when CaO was added to soils during a sludge compost decomposition study. Their data also suggest that part of the CO_2 liberated may have originated from inorganic carbonates present in the compost.

Sludge treated soils generally evolved a greater percentage of added $Ca^{14}CO_3$ than control soils (Table 10.3). This may reflect the enhanced production of organic acids or other acidic end-products of microbial metabolism in the sludge amended soils. As much as 47% of the total CO_2-C evolved from sludge treated soils was due to $CaCO_3$ dissolution. From these data, it is apparent that overestimation of sludge organic C decomposition will occur when calcareous sludges are added to soils if the data are not corrected for $CaCO_3$ dissolution.

The data in Table 10.2 indicate that the amount of residual inorganic C present in sludge amended soils incubated for 240 days was less than that predicted from the evolution of $^{14}CO_2$ from $Ca^{14}CO_3$ treated soils. This discrepancy may be due to incomplete mixing of the $Ca^{14}CO_3$ in soils or the shorter incubation period used in the ^{14}C experiment (150 days vs. 240 days). All data on the percentage of sludge organic C decomposed were corrected for inorganic C dissolution by soil analysis of residual inorganic C present after the 240 day incubation. The corrected CO_2 evolved from sludge treatments was calculated by subtracting 1) CO_2-C originating from dissolution of $CaCO_3$ and; 2) CO_2-C derived from decomposition of soil organic matter (untreated sample). (Note: Residual inorganic C in the S2 sludge treatments were used to correct all synthetic sludge treatments for $CaCO_3$ dissolution).

Decomposition of Municipal Sludges

In soils amended with municipal sludge, the amount of CO_2 evolution generally increased as the rate of sludge application increased. All four rates of the Anderson sludge followed this pattern (Figure 10.4). The 22 and 45 metric tons/ha Marion sludge rates evolved CO_2 in amounts similar to the $CaCO_3$ + 1,000 µg N/g control soil, while the 67 and 90 metric tons/ha rates evolved more CO_2 than the control soil (Figure 10.5). The CO_2 produced by all application rates of the Frankfort sludge was similar to that of the control soil (Figure 10.6). These data represent present total CO_2-C evolved.

The amount of sludge organic C decomposed was calculated for each soil + sludge sample by subtracting the CO_2-C due to $CaCO_3$ dissolution and the CO_2-C from the control soil (also corrected for $CaCO_3$ dissolution) (Table 10.4). The Anderson sludge evolved the greatest percent of sludge organic C, 24% at the 22 metric tons/ha application rate. The percentage of sludge organic C evolved generally decreased with increased application rate. Similar relationships between application rate and decomposition have been reported previously (Sommers _et al_., 1979; Agbim _et al_., 1976). The 22 and 45 metric tons/ha rates of the Marion and the 67 and 90 metric tons/ha of the Frankfort sludge treatments showed little decomposition of sludge organic C. The lack of organic C decomposition for these rates of the Marion and Frankfort sludge treatments may be related to the low organic C content (6-12%) of these sludges as opposed to liquid anaerobically digested sludges containing 14-28% organic C (Sommers _et al_., 1973). Alternatively, the organic C present may have been relatively stable. Both possibilities may result from the sludges being on sand drying beds prior to their collection where decomposition of easily decomposed materials may have occurred. The relatively low volatile solids content (31-39%) of the sludges used (Table 10.1) - liqud anaerobically digested sludges typically contain 31-51% volatile solids (Sommers _et al_., 1973) - may also reflect aerobic decomposition occurring on the drying beds. The lack of organic C deomposition in the Frankfort sludge may be due to the fact that this sludge contained approximately 1,210 µg Cd/g. Cadmium, when added as $CdCl_2$, has been shown to decrease the decomposition of leaf litter in soils (Chaney _et al_., 1978; Bhuiya and Cornfield, 1972).

Decomposition of Synthetic Sludges

To characterize the effects of metals on rates of sludge decomposition in soils, synthetic sludges were prepared and incubated in the Chalmers soil. As was found for the Anderson municipal sludge, the amount of CO_2-C evolved increased with increasing application rate for all synthetic sludge treatments (Figures 10.7 to 10.11). Some of the evolved CO_2 resulted from dissolution of added and sludge-borne $CaCO_3$ since analysis of residual $CaCO_3$ in the S2 sludge soils indicated that 72 and 90% of the initial $CaCO_3$ had dissolved at the 45 and 90 metric tons/ha rates, respectively. Application at 22 metric tons/ha for sludges containing different metal levels produced similar amounts of CO_2 (Figure 10.12). However, at the 67 and 90 metric tons/ha rates (Figures 10.13 and 10.14, respectively), the sludges with elevated metal concentrations (S3 and S4) lagged in CO_2 production initially, but by the end of 240 days, total CO_2 evolved was equal to or greater than that of sludges containing lower amounts of metals. This response may represent an initial, partial inhibition of microbes by metals followed by adaptation and recovery. The increased amount of CO_2 evolved from the 90 metric tons/ha rates of the S3 and S4 sludges may represent a stimulation of decomposition by Pb and Zn as was found by Bhuiya and Cornfield (1972); however, this is not considered to be likely since Bhuiya and Cornfield also found Ni and Cu to decrease CO_2 evolution.

For all levels of metal addition, from 46-66% of the synthetic sludge, organic C was evolved as CO_2 during 240 days of incubation (Table 10.5). Generally, except for the S0 and S1 treatments, the percentage of sludge organic C evolved as CO_2 was highest at the 22 and 90 and lowest at the 45 metric tons/ha rates. The percent organic C decomposition for the 22 and 45 metric tons/ha rates appeared to be influenced by heavy metals, decreasing from 55-66% for the S0 and S1 sludge treatments to 46-60% for the S3 and S4 sludge treatments. At the 90 metric tons/ha rate, metals apparently increased organic C decomposition, which increased from 55% for the S0 treatment to 63% in the S4 treatment. Elevated heavy metal concentrations in the digester may have inhibited digestion of the sludge material and thus resulted in the S4 sludge material containing organic C compounds more susceptible to decomposition.

The effect of metals on organic C decomposition in synthetic sludges is summarized in Figure 10.15. The metal equivalent was calculated by summing the concentrations of Cd, Ni, Cu, Pb, and Zn in each synthetic sludge. This approach should be valid since the ratio between metals in the various synthetic sludges was relatively constant. The mean decomposition for all synthetic sludges was 53.9% of the organic C added. For the majority of synthetic sludge application rates, the extent of decomposition was within 1 standard deviation of the mean. In summary, the controlling factor in decomposition of synthetic sludges did not appear to be the amount of Cd, Zn, Cu, Ni, or Pb added to soils.

Nitrification

Nitrification in the Anderson, Marion, and Frankfort sludge treated soils and in the untreated soils was essentially complete after 56 days of incubation (Table 10.8). The rate of sludge application and various differences in properties of the sludges had little effect on nitrification. These findings are notable in light of the facts that relatively large amounts of NH_4^+-N were added to the soil (250 to 1000 μg/g) and that significant amounts of heavy metals (potential nitrification inhibitors) were present in the sludges. At the above levels of NH_4^+ addition, complete nitrification can result in substantial H^+ production; however, the $CaCO_3$ present in the sludges initially, along with the added $CaCO_3$, mitigated any large pH decreases (Table 10.8).

Decreases in soil pH were observed in the study with liquid and dried municipal sludges in which soils received little or no $CaCO_3$. The West Lafayette sludge used in this study contained only 0.4% inorganic C. After 66 days of incubation, pH values had decreased as much as 1.5 units (Table 10.9) resulting in incomplete nitrification in soils treated with sludge at 60 and 100 metric tons/ha and in soils not treated with sludges (Table

10.10). Only 33 and 40% of added NH_4^+ had been nitrified at the 100 metric tons/ha rate of the dried and liquid sludge treatments, respectively. Although low pH values can markedly affect nitrification rates (Alexander, 1977), the differences in nitrification between the 15, 60, and 100 metric tons/ha rates of the liquid and dried sludge treatments is not likely caused by the relatively small differences in soil pH (pH 5.4 to 4.9 for the liquid sludge and pH 5.1 to 4.7 for the dry sludge treatments). The supernatant treatment nitrified NH_4^+ more rapidly than the other 15 metric tons/ha treatments; however, less than 110 μg N/g of NH_4^+ was added through the supernatant treatment, therefore, comparisons with the supernatant treatment are not valid. In addition, the percent of nitrified NH_4^+ consistently decreased with treatments in the following order: control > liquid sludge > dried sludge (Figure 10.16, 10.17, and 10.18). Ammonium toxicity was not considered likely since, as shown in Table 10.7, $CaCO_3$ amended control soils treated with > 1,000 μg N/g showed nitrification of all added NH_4^+ within 56 days. These data indicate that the rate of sludge addition and the form of the sludge (liquid vs. dried) influence nitrification. Reasons for these effects will be discussed later.

Nitrification of added NH_4^+ was incomplete in the synthetic sludge amended soils after 240 days of incubation. At the 90 metric tons/ha rate of the S3 and S4 sludge treatments, only 6 and 0%, respectively, of the added NH_4^+ was nitrified (Table 10.8). This delay in nitrification was partly due to heavy metal toxicity since the percent of NH_4^+ nitrified decreased with increasing amounts of heavy metals added to soils. That is, nitrification was depressed in soils treated with the 67 and 90 metric tons/ha rates of the S3 and S4 sludges as compared to the S0 (no metals) treatments. Heavy metal inhibition of nitrification has been reported in a previous study using soils amended with synthetic sludges (Silviera, 1976). Generally, the percent of NH_4^+ that was nitrified decreased both as the rate of application and the concentration of heavy metals increased (Table 10.9). Examination of the total metals added at each sludge treatment provides information on the relative influence of sludge application rate and heavy metal concentrations. The 90 metric tons/ha rate of the S1, the 45 metric tons/ha rate of the S2, and the 22 metric tons/ha rate of the S3 sludge all added approximately equal amounts of heavy metals to soils and yet the S1 treatment nitrified only 59% of the NH_4^+ while the S2 and S3 treatments nitrified 100 and 98%, respectively. Likewise, although heavy metal content was equal, the 90 metric tons/ha rate of the S3 sludge and the 45 metric tons/ha rate of the S4 sludge nitrified 6 and 100% of added NH_4^+-N, respectively. These differences in nitrification may be due to immobilization of inorganic N at the high application rate of sludge.

Although heavy metals appear to influence nitrification in soils treated with the synthetic sludges, it should be noted that the relatively high Cd concentration of the Frankfort sludge (1,210 μg/g) had no effect on nitrification. Furthermore, heavy metals were not solely responsible for delays in nitrification since application of the S0 sludge at 90 metric tons/ha, which contained no added metals, showed only 6% nitrification of added NH_4^+ by 56 days. The low nitrification in this treatment at 56 days (with substantial NH_4^+ present, Table 10.8) is evidence that heavy metals were not wholly responsible for the delayed nitrification in the synthetic sludges with varying metal concentrations.

The absence of delayed nitrification in soils receiving the Anderson, Marion, and Frankfort sludges, particularly at 90 metric tons/ha rates, contrasts sharply with previous reports on nitrification in sludge-amended soils (Premi and Cornfield, 1969, 1971; Ryan _et al._, 1973; Wilson, 1977a), and with the data from the synthetic sludge amended soils. (Data from the dried vs. liquid municipal sludge study cannot be compared directly, due to the low soil pH in sludge treated soils.) The lack of nitrification inhibition in the high rates of the municipal sludges may be due, in part, to differences in handling and properties of the bed-dried sludges used in this study and the liquid or flash-dried sludges employed in studies reported in the literature. The bed-dried sludges from Anderson, Marion, and Frankfort have relatively low organic C, total N, and volatile solids compared to liquid sludges (Sommers _et al._, 1973) and the synthetic sludges (Table 10.1). These properties indicate that the bed-dried sludges have probably undergone considerable decomposition and leaching while on the drying beds. Since toxic organic compound(s) in the sludge has (have) been proposed as the agent(s) responsible for nitrification inhibition at high rates of sludge application (Premi and Cornfield, 1969; Ryan _et al._, 1973), it is possible that the sludges used in this study underwent sufficient decomposition on the drying beds such that the organic compound(s) responsible for inhibition of nitrification was (were) degraded. It should be noted that the study conducted with dried and liquid sludge involved a three week drying period whereby minimal changes occurred in organic C. Several organic compounds are known to inhibit nitrification (i.e., ethanol, acetone, sodium and potassium azide) some of which are industrially significant and may enter sludge treatment systems (ethyl acetate, N-propanol, methanol, acetone) (Hockenbury and Grady, 1977; Bundy and Bremner, 1973). If organic compounds in the sludge are responsible for the delayed nitrification, the compounds are likely of microbial origin since delayed nitrification was also observed in the synthetic sludge treatments where yeast cells supplied all the available organic C.

Another possible explanation to the lack of nitrification inhibition at high application rates of municipal sludges also relates to organic material in the sludges. Sludge materials enriched in organic carbon reportedly reduce nitrification during sludge treatment (Steinquist _et al._, 1974; Weng and Molof, 1974) Apparently, high levels of organic carbon and N stimulate heterotrophic microbial populations, thereby creating an unfavorable balance of heterotrophic and autotrophic (i.e., nitrifiers) microorganisms. The dried municipal sludges (Anderson, Marion, and Frankfort) used in this study contained less organic C than the synthetic sludges and therefore would not have stimulated heterotrophic microbial populations to as great an extent as the synthetic sludges (or a sludge with a greater amount of organic C) and, therefore, would not adversely affect the nitrifying populations.

Mineralization of Organic Nitrogen

In general, the inorganic N concentrations in soil amended with the Anderson, Marion, and Frankfort sludges decreased initially and then increased with time (Figure 10.19). The initial decrease in inorganic N was likely due to immobilization which has been observed in a similar study (Terry, 1976). The 67 and 90 metric tons/ha rates of the Anderson, Marion, and Frankfort sludge treatments showed net mineralization of organic N after

240 days of incubation with values ranging from 13-64% of added sludge organic N (Table 10.8). The 22 and 45 metric tons/ha rates showed either net immobilization or slight mineralization. The percent of organic N mineralized in these sludge-treated soils generally increased with application rate. This finding contrasts with previous reports, wherein the percent of sludge organic N mineralized either decreased with increasing application rate (Premi and Cornfield, 1971; Ryan et al., 1973) or remained relatively constant (Epstein et al., 1978). No relationship was observed between organic N added and percent organic N mineralized.

Although comparison of data from the study using liquid and dried sludge with data from the other municipal sludges is complicated by the low soil pH in soils treated with sludge without $CaCO_3$ (Alexander, 1977), comparison of treatments within the study are valid. All rates of the dried sludge treatments consistently mineralized a greater percentage of the sludge organic N than the corresponding rates of the liquid sludge treatments. Soils treated with dried sludge had similar amounts of N mineralization (31 to 28% of organic N added) at the 15 to 100 metric tons/ha application rates, while N mineralization in the liquid sludge treated soils increased from 21 to 28% with increased application rate (Table 10.9). These differences in mineralization between treatments may be a result of differences in microbial activity and concomitant immobilization or the ease of decomposition of the sludge organic N (i.e., the dried sludge had undergone some aerobic decomposition which may have converted complex organic N compounds into more readily degradable compounds).

In soils amended with synthetic sludges, inorganic N concentrations initially decreased (immobilization), but gradually increased with time (Figures 10.20 and 10.21). The initial decrease in inorganic N was more pronounced in the synthetic than the municipal sludge treated soils. However, since the synthetic sludge-treated soils supported greater microbial activity (based on CO_2 evolution data presented earlier), more immobilization of N is anticipated in soils containing the synthetic sludges. The percent of synthetic sludge organic N mineralized was variable, but generally increased with increased application rates (Table 10.8). Since the mineralization values in the metal amended sludge treatments were not consistently higher or lower than the synthetic sludge containing no added metals (S0), definite conclusions regarding the effects of heavy metals on sludge N mineralization are not evident. It was noted that at the 90 metric tons/ha rate, mineralization of sludge organic N increased with sludge heavy metal concentration. However, unexplained decreases in inorganic N content (immobilization) in the 67 and 90 metric tons/ha rates of the S0 treatment (Figure 10.22) late in the study, may have influenced mineralization. Heavy metals have been reported to both inhibit and stimulate mineralization of organic N in soils (Premi and Cornfield, 1971; Premi, 1970; Liang and Tabatabai, 1977).

The 90 metric tons/ha rate of the S0, S3 and S4 sludge treatments had the greatest microbial activity (as determined by CO_2 evolution) and likewise mineralized the highest percentages of sludge organic N (Table 10.8). The correlation between CO_2 release (microbial acitivity) and organic N mineralization in soils has been noted (Alexander, 1977).

Extractability of Heavy Metal

A linear relationship was found between added and DTPA extractable metals for all sludge-treated soils (Table 10.11, Fig. 10.23 to 10.26). Correlation coefficients between added and DTPA extractable metals were significant ($p = .05$) for all metals when all sludge treatments were combined and when synthetic and municipal sludges were considered separately. Except for Zn and Ni in the Marion sludge treatments, each individual sludge also had a significant correlation ($p = .05$) between added and DTPA extractable metals. The poor correlation between added and DTPA extractable Zn and Ni in the Marion sludge treatments may have been due to the pH and/or $CaCO_3$ content of the soil in these treatments. The absence of a decrease in soil pH with increased rates of the Marion sludge was due to the greater amount of $CaCO_3$ present in the 67 and 90 metric tons/ha rates. The greater amount of $CaCO_3$ added offset the H^+ produced during nitrification of the NH_4^+ added at the 67 and 90 metric tons/ha rates. The pH or $CaCO_3$ effect on metal extraction will be discussed in more detail later.

The significant correlations obtained between added and DTPA extractable metals for the synthetic and municipal sludges indicate that the metals in all sludge amended soils are behaving similarly and therefore, the synthetic sludges may be valuable in studying the chemistry of metals in sludge amended soils. In addition, the significant correlation between added and DTPA extractable metals allows the prediction of DTPA extractable metals from the metal content of the sludge, realizing that additional soil types should be studied before any definitive generalizations can be made.

In general, the amount of DTPA extractable metals increased with time in all sludge treated soils (Figures 10.23, to 10.26; Table 10.12). Analysis of DTPA extractable metals for all sludge treatments combined, indicated that only DTPA-Cd and -Zn had significant differences ($p = .05$) between day zero and day 240 (Table 10.13, Figures 10.24 and 10.25). Although not statistically significant, Cu and Ni also increased over time, as evidenced in Figures 10.23 and 10.26, and in the slopes of the lines describing the regression equations of DTPA extractable and added metals (Table 10.14). The increase in extractability of Cd and Zn with time contrasts with the reversion phenomenon discussed by Chaney (1973). The increase in Cd and Zn extractability could be due to the decrease in soil pH caused by nitrification of added NH_4^+ in the sludge amended soils (Table 10.8). Lindsay (1972) has noted that large increases in metal solubility occur with relatively small decreases in soil pH. In addition to soil pH, decomposition of sludge organic C could result in release of metals formerly bound in organic complexes. Since substantial amounts of heavy metals are associated with the sludge light fraction decomposition of sludge organic materials could increase the extractability of metals in the soil. However, since the percent of organic C decomposed generally decreased with increasing sludge application rate and since the percent of added metals extracted with DTPA increased with increasing application rate (Table 10.12), the release of metals by decomposition of organic matter does not appear to be a major factor in the increase of extractable metals with time. This point is particularly evident in the Marion and Frankfort sludge treated soils in which only zero to 19% of the organic C was decomposed, yet from 20 to 70% and 31 to 71% of added Cd and Cu, respectively, were extractable.

Although decomposition of organic material apparently had little effect on metal extractability, the amount of sludge organic C added appeared to influence metal extractability. The use of synthetic sludges enabled the addition of the same amount of heavy metals at different rates of sludge addition (i.e., organic C added varied while metal added was constant). For example, the following synthetic sludge-application rate combinations added similar amounts of each metal to the soil - S2 @ 90 metric tons/ha, S3 @ 45 metric tons/ha, and S4 @ 22 metric tons/ha. The results indicate that Ni extracted by DTPA decreased from 43 to 20% and Zn extracted decreased from 42 to 29% in the S2, S3 and S4 treatments. Although the total amount of metals added to the soil was the same, the soils that received the high application rates of sludge had more DTPA available Zn and Ni than the sludges containing higher metal levels, but added to soil at a lower rate. The increased DTPA extractability of Ni and Zn at high synthetic sludge application rates may result from the large amount of organic matter added at these rates. With the large increase in organic matter and subsequent increase in microbial activity, microbial by-products may have temporarily solubilized metals and increased their extractability with DTPA. Alternatively, the lower soil pH in the high application rate treatments (Table 10.8) may have increased metal solubility.

The percentage of added sludge metals extracted from the soil with DTPA was variable depending on the sludge treatment (Table 10.12). Values for the municipal and synthetic sludges ranged from < 1 to 71% for Cu, 31 to 75% for Cd, 15 to 55% for Zn, and < 1 to 59% for Ni after 240 days of incubation. As previously discussed, the percentage of DTPA extractable metals generally increased with increasing rate of sludge application. The decrease in percentage of added metals extracted with DTPA from zero to 240 days of incubation occurred primarily in the 22 metric tons/ha rates and may indicate biological or physical immobilization (i.e., microbial assimilation, occlusion) of metals at the relatively low metal additions.

SUMMARY

Although differences in sludge properties (i.e., volatile solids, heavy metals, etc.) prevent direct comparison of the decomposition of municipal and synthetic sludges, the synthetic sludges provide valuable information on the effects of metals on sludge decomposition. Data from the synthetic sludges show that high levels of metals may produce a temporary inhibition of organic C decomposition. In addition, high application rates of metal containing sludges stimulated instead of decreased organic C decomposition. This result may also be due to metals altering the composition of the sludge produced during anaerobic digestion.

Decomposition of municipal sludges was low relative to the synthetic sludge and to values reported by other researchers (e.g., Miller, 1974; Agbim and Sabey, 1977; Sommers et al., 1976). The reduced decomposition was apparently caused by drying the sludge on drying beds where the sludge underwent decomposition resulting in a product that resists further decomposition. In addition, data obtained from incubation of calcareous sludges in soil may be an overestimation of the amount of decomposition occurring if CO_2 evolved is measured and not corrected for $CaCO_3$ dissolution.

In conclusion, metal concentrations in sludges are a minor factor in determining the amount of sludge organic C decomposition in soils. Nitrification in synthetic and some municipal sludge amended soils was inhibited by low soil pH, high rates of sludge application, and elevated concentrations of heavy metals. However, nitrification in some municipal sludges was not affected by either rate of application or heavy metal concentration. Whereas, heavy metal toxicity and pH effects on nitrification have been documented, the cause for nitrification inhibition at high application rates (in the absence of heavy metals) is unknown but could be related to depressed O_2 during rapid decomposition of sludge organic matter. This is supported by the fact that sludges resistant to decomposition nitrified more rapidly at high application rates than sludges more susceptible to decomposition.

Mineralization of sludge organic N in all sludge amended soils was variable depending on the sludge, but generally increased with application rate. Heavy metal effects on mineralization of sludge organic N were not well defined. Therefore, although high application rates of sludge may mineralize a greater percentage of the organic N, leaching of NO_3^- may not be a concern since nitrification of NH_4^+ to NO_3^- may not occur as rapidly.

Heavy metals extracted with DTPA were directly related to the amount of sludge-borne metals added to the soil. Similar correlations between added and DTPA extractable metals were obtained with synthetic or municipal sludges, indicating that the metals in the synthetic sludges were behaving similarly to those in the municipal sludges. This suggests that synthetic sludges offer promise for studies of metal chemistry in sludge amended soils enabling the preparation of sludges varying in only one parameter.

The amount of DTPA extractable Cd and Zn generally increased with incubation time. Decreases in soil pH, particularly at high rates of sludge application may, in part, be responsible for the increases in metal extractability.

Table 10.1. Properties of synthetic and municipal sludges.

Component	Synthetic sludges					Municipal sludges		
	S0	S1	S2	S3	S4	Anderson	Marion	Frankfort
					%			
Volatile solids	50.0	48.5	49.8	47.7	47.5	34.2	38.8	31.2
Inorg. N	0.91	1.11	0.99	0.99	0.95	0.068	0.035	0.137
Org. N	1.84	1.85	2.19	1.82	2.15	0.665	0.666	1.421
Org. C	21.9	22.3	22.5	23.8	22.1	12.98	6.46	12.41
Inorg. C	2.2	2.2	2.5	2.4	2.0	2.17	5.57	1.32
					µg/g			
Cu	46	687	1,346	2,571	5,130	1,200	450	1,330
Zn	437	1,739	3,263	6,300	10,286	8,420	7,160	2,800
Cd	7	128	268	524	981	310	370	1,210
Pb	ND*	688	1,324	2,604	4,969	1,070	1,520	3,480
Ni	16	611	1,280	2,702	4,671	2,200	120	600

* ND signifies not detectable

Table 10.2. Initial and final inorganic C concentrations in a Chalmers soil treated with sludge and incubated for 150 days.

Sludge	Rate or amendment	Inorganic C		Inorganic C evolved as CO_2-C	
		Initial	Final	Direct measurement[a]	^{14}C measurement
	metric tons/ha	-- mg C/g soil ---		------- % of added C -------	
Anderson	22	1.229	.256	79	59
	90	1.200	.092	92	72
Marion	22	1.232	.295	76	67
	90	2.249	.678	70	-
Frankfort	22	1.228	.220	82	62
	90	1.197	.090	93	84
None	CLN*	1.238	.261	79	60
	CHN	1.238	.029	98	69
	C	1.238	.418	66	52
	NA	0.111	.014	87	-

[a] 240 day expt.

* CLN, 9.49 mg $CaCO_3$/g, 250 µg $(NH_4)_2SO_4$-N/g; CHN, 9.49 mg $CaCO_3$/g, 1,000 µg $(NH_4)_2SO_4$-N/g; C, 9.49 mg $CaCO_3$/g; NA, no amendment

Table 10.3. Contribution of $CaCO_3$ dissolution to total CO_2 evolved and pH values from sludge treated and control soils after 150 days incubation.

Sludge	Rate or amendment	$CaCO_3$-C added		CO_2-C evolved			CO_2-C from		pH	
		Indigenous	$Ca^{14}CO_3$	Total	$CaCO_3$*	Organic	% of added	% of total evolved	Initial	Final
	metric tons/ ha	---- mg/g ----								
Anderson	22	0.211	0.907	1.774	0.664	1.110	59	37	7.7	7.4
	90	0.828	0.261	2.659	0.788	1.871	72	30	7.4	6.2
Marion	22	0.553	0.568	1.615	0.753	0.862	67	47	7.7	7.3
Frankfort	22	0.127	0.990	1.639	0.693	0.946	62	42	7.8	7.4
	90	0.500	0.586	2.528	0.914	1.614	84	36	7.3	6.2
None+	CLN		1.127	1.695	0.675	1.020	60	40	7.7	7.6
	CHN		1.127	1.818	0.772	1.046	69	43	7.8	6.2
	CC		1.127	1.692	0.586	1.106	52	35	8.2	8.0
	NA			.969						

* assuming indigenous and added $CaCO_3$ dissolve at the same rate as $Ca^{14}CO_3$

+ CLN, 9.49 mg $CaCO_3$/g, 250 μg $(NH_4)_2SO_4$-N/g; CHN, 9.49 mg $CaCO_3$/g, 1,000 μg $(NH_4)_2SO_4$-N/g; C, 9.49 mg $CaCO_3$/g; NA, no amendment.

Table 10.4. Evolution of CO_2-C from a Chalmers soil amended with municipal sludges and incubated for 240 days.

Sludge	Rate or amendment	Final pH	Inorg. C dissolved*	CO_2-C evolved			Sludge org. C evolved	
				Total	$CaCO_3$	Org. C	mg/g$^+$	% of‡ added
	metric tons/ha		% of added	--------	mg/g	--------		
Anderson§	22	6.4	79	2.299	0.880	1.419	0.289	22.3d
	45	6.4	(83.5)	2.450	0.930	1.520	0.472	18.2cd
	67	6.2	(87.7)	2.479	0.977	1.502	0.522	14.1bcd
	90	5.9	92	2.694	1.025	1.669	0.763	14.7cd
Marion§	22	6.5	76	1.941	0.847	1.094	-0.04	-
	45	6.4	(74.0)	1.849	0.824	1.025	-0.02	-
	67	6.4	(72.0)	2.341	1.203	1.138	0.158	8.2abc
	90	6.4	70	2.639	1.462	1.177	0.271	10.5abcd
Frankfort§	22	6.6	82	2.051	0.914	1.138	0.008	0.6a
	45	6.4	(86.0)	2.054	0.958	1.096	0.048	1.9ab
	67	6.1	(89.5)	1.875	0.997	0.878	-0.102	-
	90	5.7	93	1.892	1.036	0.856	-0.050	-
None	250 µg N/g (22)**	6.8	79	2.010	0.880	1.130	-	-
	(45)		(86.0)	(2.006)	(0.958)	(1.048)	-	-
	(67)		(91.7)	(2.002)	(1.022)	(0.980)	-	-
	1,000 µg N/g (90)	5.8	98	1.998	1.092	0.906	-	-
	-	6.7	66	1.879	0.590	1.289	-	-
	NA	5.6	87	1.083	0.097	0.986	-	-

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* Based on data presented in Table 10.2.

+ CO_2-C from organic C for treatment minus control.

‡ Values followed by the same letter are not significantly different ($p = .05$) (Duncan's test).

§ Except for Marion 67 and 90 metric tons/ha treatments, soils were adjusted to contain 1.114 mg $CaCO_3$-C/g.

** Treatments serve as controls for 22, 45, 67 or 90 mt/ha sludge additions (shown in parenthesis).

Table 10.5. Evolution of CO_2-C from a Chalmers soil amended with synthetic sludges and incubated for 240 days.

Sludge	Rate or amendment	Final pH	CO_2-C evolved			Sludge organic C		
			Total	$CaCO_3$*	Org C	Added	Evolved†	Evolved‡
	metric tons/ha		----------------	----------------	mg/g	----------------	----------------	-- % ---
S0	22	6.6	3.200	0.899	2.301	2.19	1.42	64.8d
	45	6.4	4.200	0.844	3.356	4.38	2.40	54.7bc
	67	6.0	5.605	0.984	4.621	6.57	3.60	54.8bc
	90	5.9	6.992	1.080	5.912	8.76	4.82	55.0bc
S1	22	6.5	3.243	0.899	2.344	2.23	1.46	65.7d
	45	6.2	4.293	0.844	3.449	4.46	2.49	55.9bc
	67	6.0	5.661	0.984	4.677	6.69	3.66	54.7bc
	90	5.8	7.131	1.080	6.051	8.92	4.96	55.6c
S2	22	6.8	3.195	0.899	2.296	2.25	1.42	62.9d
	45	6.3	4.210	0.844	3.366	4.50	2.41	53.5bc
	67	5.9	5.648	0.984	4.664	6.75	3.64	53.9abc
	90	5.8	7.840	1.080	6.760	9.00	5.67	63.0d
S3	22	6.6	3.104	0.899	2.205	2.38	1.33	55.7c
	45	6.2	4.063	0.844	3.219	4.76	2.26	47.5ab
	67	5.8	5.720	0.984	4.736	7.14	3.71	52.0abc
	90	5.9	7.741	1.080	6.661	9.52	5.57	58.5cd
S4	22	6.9	3.111	0.899	2.212	2.21	1.33	60.2d
	45	6.6	3.812	0.844	2.968	4.42	2.01	45.5a
	67	5.8	5.664	0.984	4.680	6.63	3.66	55.2c
	90	5.8	7.757	1.080	6.677	8.84	5.59	63.2d
None	250 µg N/g	6.8(22)§	2.010	1.130	0.880	-	-	-
		(45)	(2.006)**	(1.048)	0.958	-	-	-
		(67)	(2.002)	(0.980)	1.022	-	-	-
	1,000 µg N/g	5.8(90)	1.998	0.906	1.092	-	-	-

Table 10.5. (Cont.)

* Based on initial and residual CO_3-C in the S2 sludge treated soil. See soils adjusted to contain 1.14 mg $CaCO_3$-C/g.

+ CO_2-C from organic C for treatment minus control.

‡ Values followed by the same letter are not significantly different ($p = .05$) with a Duncan's multiple range test.

** Interpolated values in parenthesis.

§ Treatments serve as controls for 22, 45, 67 or 90 metric tons/ha sludge additions (shown in parenthesis).

Table 10.6. Equations describing cumulative CO_2 evolution from and priming effect ratios of municipal and synthetic sludge amended soils incubated for 240 days.

Sludge	Equation[+]	R^2	Priming effect ratio[*]
Anderson	$y = 0.12\ x + 2.18$	.93	1.16
Marion	$y = 0.26\ x + 1.55$	.83	.82
Frankfort	$y = -0.06\ x + 2.13$	.73	1.13
S0	$y = 1.28\ x + 1.80$	.99	.96
S1	$y = 1.30\ x + 1.82$	.99	.97
S2	$y = 1.54\ x + 1.38$	.97	.73
S3	$y = 1.56\ x + 1.27$	.98	.68
S4	$y = 1.58\ x + 1.14$	.96	.61

[+] $y = CO_2$-C evolved, mg/g; x = rate of sludge application, g/100 g soil

[*] ratio of b/b', where b = CO_2-C calculated from above equation for treatment at 0 rate of sludge application (i.e., y intercept); b = CO_2-C evolved from control soil + $CaCO_3$ = 1.88 mg CO_2-C/g.

Table 10.7. Nitrification and N mineralization values for a Chalmers soil amended with municipal and synthetic sludges and incubated for 56 days.

Treatment	Rate or amendment	NH_4^+	NO_2^- + NO_3^-	Sum	% of NH_4^+ nitrified*	Sludge org N mineralized +
	metric tons/ ha	-------	μg/g	-------	---------- %	----------
Anderson	22	5	259	264	98	-
	45	8	488	496	98	2
	67	10	713	723	98	27
	90	18	935	953	98	28
Marion	22	6	288	294	97	-
	45	9	525	534	98	30
	67	11	760	771	98	59
	90	9	999	1008	99	46
Frankfort	22	8	291	299	95	-
	45	6	536	542	98	8
	67	5	781	786	99	24
	90	13	994	1007	98	22
S1	22	7	256	263	96	-
	45	9	444	453	98	-
	67	556	98	654	0	16
	90	819	57	876	-	22
S2	22	10	257	267	95	-
	45	11	469	480	97	-
	67	577	100	677	11	2
	90	850	69	919	-	16
S3	22	8	258	266	96	-
	45	155	284	439	62	-
	67	616	53	669	3	3
	90	826	36	862	-	10
S4	22	9	273	282	96	-
	45	221	241	462	47	-
	67	682	36	718	-	12
	90	924	31	955	-	13
S0	22	6	247	253	97	-
	45	12	441	453	97	1
	67	47	595	642	92	1
	90	792	105	897	6	9
Control‡	22	9	286	295	95	
	(45)§			(540)		
	(67)			(785)		
	90	12	1018	1030	96	

Table 10.7. (Cont.)

‡ Sludges added 250 and 1000 μg N/g soil at 22 and 90 metric tons/ha, respectively.

§ Values in parenthesis are interpolated.

* Ammonium nitrified - % of added NH_4^+-N recovered as NO_2^- + NO_3^--N

+ Organic N mineralized - % of added organic N recovered as NH_4^+ + NO_2^- + NO_3^--N

Table 10.8. Nitrification and N mineralization values for a Chalmers soil amended with municipal and synthetic sludges and incubated for 240 days.

Treatment	Rate or amendment	NH_4^+	$NO_3^- + NO_2^-$	Sum	NH_4^+ Nitrified	Org. N mineralized⁺	pH Day 40	pH Day 240
	metric tons/ ha	--------	µg/g	--------	----------	% ----------		
Anderson	22	3	359	362	99	-	7.0	6.4
	45	4	542	546	99	-	6.9	6.4
	67	4	773	777	99	23	6.6	6.2
	90	21	997	1018	100	31	6.5	5.9
Marion	22	1	347	348	99	-	7.2	6.5
	45	4	574	578	99	12	6.9	6.4
	67	4	838	842	99	64	7.0	6.4
	90	6	1046	1052	99	41	7.0	6.4
Frankfort	22	1	376	377	99	-	7.3	6.6
	45	5	595	600	97	7	7.0	6.4
	67	3	802	805	99	13	6.5	6.1
	90	13	1006	1019	98	14	6.0	5.7
S1	22	1	351	352	99	-	6.6	6.5
	45	4	576	580	99	14	6.5	6.2
	67	6	802	808	99	33	6.5	6.0
	90	308	609	917	59	20	6.6	5.8
S2	22	1	372	373	99	6	7.1	6.8
	45	2	601	603	100	12	6.7	6.3
	67	5	811	816	99	14	6.7	5.9
	90	231	927	1158	72	38	6.5	5.8
S3	22	4	367	371	98	-	6.8	6.6
	45	4	512	516	99	-	6.6	6.2
	67	14	754	768	82	29	6.3	5.8
	90	758	376	1134	6	41	6.9	5.9

Table 10.8. (Cont.)

Treatment	Rate or amendment	NH_4^+	$NO_3^- + NO_2^-$	Sum	NH_4^+ nitrified*	Org. N mineralized+	pH	
							Day 40	Day 240
	metric tons/ ha	--------	µg/g	--------	----------	% ----------		
S4	22	1	386	387	103	-	6.9	6.5
	45	2	564	566	100	5	6.6	6.1
	67	228	600	828	63	14	6.4	5.8
	90	926	327	1253	-	43	6.4	5.8
SO	22	2	371	373	95	-	7.1	6.6
	45	3	580	583	99	18	6.9	6.4
	67	4	804	808	99	20	6.5	6.0
	90	13	949	962	99	9	6.6	5.9
Control#	22	5	363	368			6.5	6.4
	(45)§			(608)				
	(67)			(847)				
	90	14	1073	1087			6.0	5.8

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* NH_4^+ nitrified - % of added NH_4^+-N recovered as $NO_2^- + NO_3^-$-N after 240 days

+ Org. N mineralized - % of added organic N recovered as $NH_4^+ + NO_2^- + NO_3^-$-N after 240 days

# Sludges added 250 and 1000 µg N/g soil at 22 and 90 metric tons/ha, respectively.

§ Values in parenthesis are interpolated.

Table 10.9. Nitrogen mineralization and pH values for a Chalmers soil amended with liquid and dried West Lafayette sludge and sludge supernatant and incubated for 66 days.

Treatment+	Rate or amendment	NH_4^+	$NO_3^- + NO_2^-$	Sum	Sludge Org N Mineralized*		pH	
							Day 6	Day 66
	metric tons/ ha	-------------	µg/g	---------------		-- % --		
Control	0	0	67	67			6.1	5.6
	15	2	155	157			5.9	5.1
	60	46	390	436			5.8	4.3
	100	341	343	684			5.7	4.4
Liquid sludge	15	2	215	217	63	20.9a	6.4	5.4
	60	120	605	725	255	24.5ab	6.2	4.8
	100	381	741	1122	469	27.5bd	6.4	4.9
Dried sludge	15	4	219	223	67	30.6c	6.1	5.1
	60	152	551	703	248	28.9cd	6.2	4.7
	100	454	635	1089	392	27.9cd	6.2	4.9
Supernatant	15	2	108	110	0	0	6.3	5.5

* Org. N mineralized - % of organic N added recovered as $NH_4^+ + NO_2^- + NO_3^-$-N after 66 days. Numbers followed by the same letters are not significantly different at the 0.01 level by t test.

+ The sludges added 110, 430, and 704 µg N/g soil at 15, 60, and 100 metric tons/ha, respectively.

Table 10.10. Nitrification of NH_4^+ in a Chalmers soil amended with liquid and dried West Lafayette sludge and the sludge supernatant.

Treatment+	Rate or amendment	Incubation time, days							
		1	5	12	19	28	38	52	66
	metric tons/ ha	---------------- % of NH_4^+-N nitrified* ----------------							
Control	15	4	10	25	59	96	101	104	101
	60	1	2	3	18	47	71	85	89
	100	1	-1	5	12	30	44	48	49
Liquid sludge	15	3	10	6	40	95	99	100	100
	60	0	0	-15	2	16	52	72	71
	100	0	-16	-16	-25	-9	19	43	40
Dried sludge	15	2	-6	-17	15	74	96	96	98
	60	0	-2	-26	-36	-18	23	56	62
	100	0	1	-11	-22	-16	10	37	33
Supernatant	15	3	7	45	84	104	100	102	98

+ The sludges added 110, 430, and 704 μg N/g soil at 15, 60, and 100 metric tons/ha, respectively.

* % of added NH_4^+-N recovered as $NH_4^+ + NO_2^- + NO_3^-$-N after 66 days.

Table 10.11. Correlation coefficients (r) for the relationship between added and DTPA extractable metals in Chalmers soil amended with synthetic and municipal sludges and incubated for 240 days.

Sludge(s)	Time	r			
		Metal			
		Cu	Cd	Zn	Ni
	- days -				
All	0	.70*	.97*	.91*	.94*
	240	.97*	.98*	.88*	.97*
Synthetic only	0	.90*	.94*	1.00*	.99*
	240	.99*	1.00*	.99*	.99*
S1	0	.99*	1.00*	1.00*	1.00*
	240	1.00*	.99*	.97*	1.00*
S2	0	.99*	1.00*	.97*	1.00*
	240	1.00*	1.00*	1.00*	1.00*
S3	0	.96*	1.00*	1.00*	.99*
	240	1.00*	1.00*	1.00*	1.00*
S4	0	1.00*	.98*	1.00*	.99*
	240	1.00*	1.00*	.99*	1.00*
Municipal only	0	.97*	.99*	.75*	.99*
	240	.97*	.98*	.71*	.91*
Anderson	0	.97*	1.00*	.99*	1.00*
	240	.99*	.99*	.97*	.96*
Marion	0	.98*	1.00*	.99*	.77
	240	1.00*	.98*	.89	.89
Frankfort	0	1.00*	1.00*	.99*	1.00*
	240	.99*	.99*	.98*	.98*

* r values are significant at 0.05 level.

Table 10.12. Percent of added metal extracted by DTPA in Chalmers soil amended with synthetic and municipal sludges and incubated for 240 days.

Sludge	Rate	Metal							
		Cu		Cd		Zn		Ni	
		Time, days							
		0	240	0	240	0	240	0	240
	metric tons/ha	%*							
S1	22	1	21	31	31	17	15	18	1
	45	1	26	32	44	26	50	24	18
	67	4	29	35	46	30	44	31	31
	90	7	29	35	43	28	42	32	35
S2	22	1	1	31	35	21	25	23	5
	45	3	30	35	48	30	39	30	29
	67	7	33	38	51	28	41	36	39
	90	7	34	36	50	24	42	38	43
S3	22	16	19	37	48	34	18	32	15
	45	19	36	48	53	35	40	40	37
	67	18	39	53	55	38	47	44	47
	90	15	38	58	53	36	48	54	50
S4	22	10	28	34	52	38	29	40	20
	45	8	38	26	62	33	52	45	46
	67	8	44	41	66	34	55	54	56
	90	8	47	44	61	35	50	61	59
Anderson	22	23	19	40	53	40	26	7	1
	45	51	33	45	55	44	29	10	7
	67	40	28	47	61	36	34	10	18
	90	51	33	47	70	39	49	12	31

Table 10.12. (Cont.)

Sludge	Rate	Metal							
		Cu		Cd		Zn		Ni	
		Time, days							
		0	240	0	240	0	240	0	240
	metric tons/ ha	%*							
Marion	22	23	50	8	25	13	9	1	1
	45	23	70	10	26	13	11	1	1
	67	38	70	10	22	11	11	1	1
	90	47	71	11	20	11	7	1	1
Frankfort	22	1	17	30	41	40	4	5	1
	45	12	24	45	47	31	19	12	1
	67	13	34	48	75	25	38	12	1
	90	16	40	53	72	24	52	14	1

* % of added metals extracted by DTPA corrected for the unamended control soil

Table 10.13. Equations describing the relationship between added and DTPA extractable metals in a Chalmers soil amended with synthetic and municipal sludge and incubated for 240 days.

Metal	Time	Equation*	R
	-days-		
Cu	0	$y = .24x + 2.33$	.70
	240	$y = .40x - 0.27$	.97
Cd‡	0	$y = .48x - 0.17$	.97
	240	$y = .70x - 1.60$	.98
Zn‡	0	$y = .33x + 3.17$	.91
	240	$y = .44x - 2.23$	.88
Ni	0	$y = .53x - 2.64$	.94
	240	$y = .55x - 4.24$	.97

* y = DTPA extractable metal, µg/g; x = Added metal, µg/g

‡ Equations for 0 and 240 days were significant different at 0.05 level.

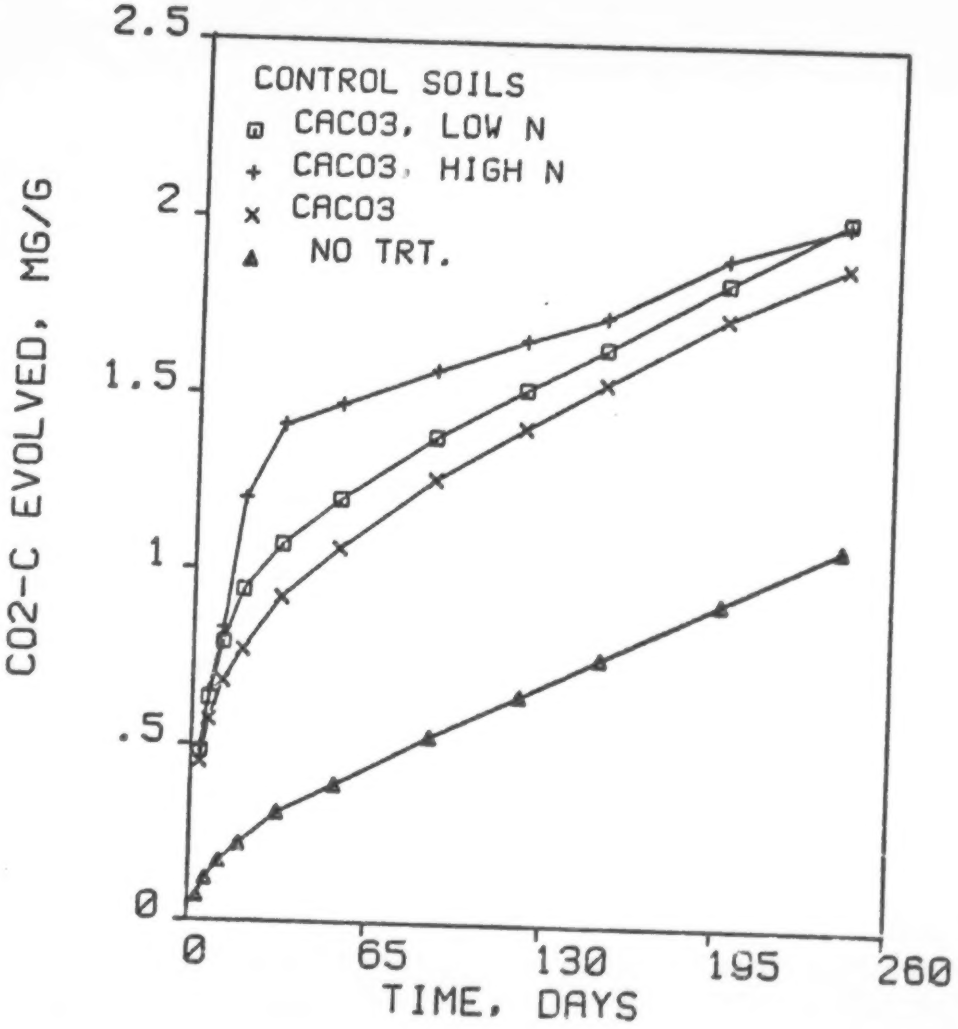

Figure 10.1. Cumulative total CO_2-C evolved from a Chalmers sil soil amended with $CaCO_3$ and N.

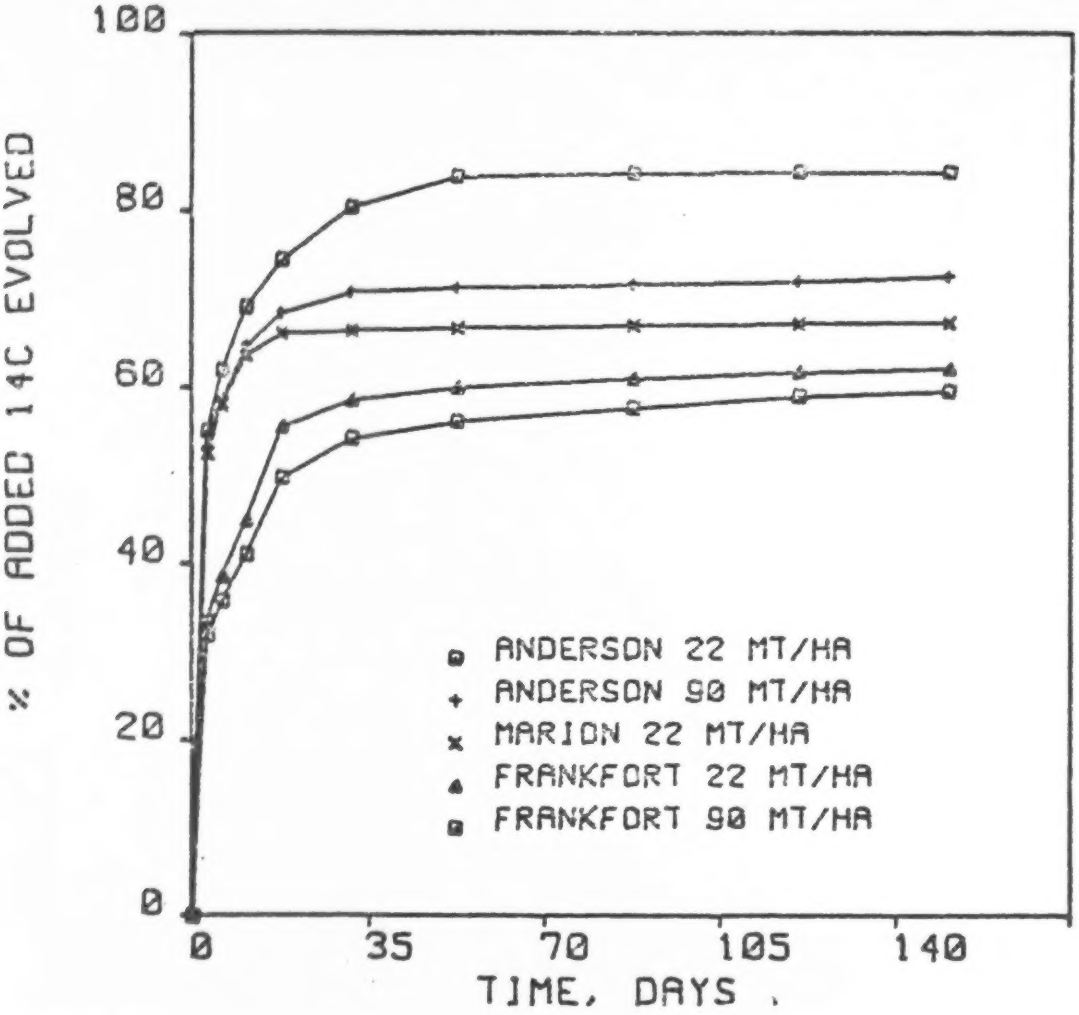

Figure 10.2. Percent of ^{14}C evolved as $^{14}CO_2$ from a Chalmers soil amended with $Ca^{14}CO_3$ and Anderson, Marion, or Frankfort sludges.

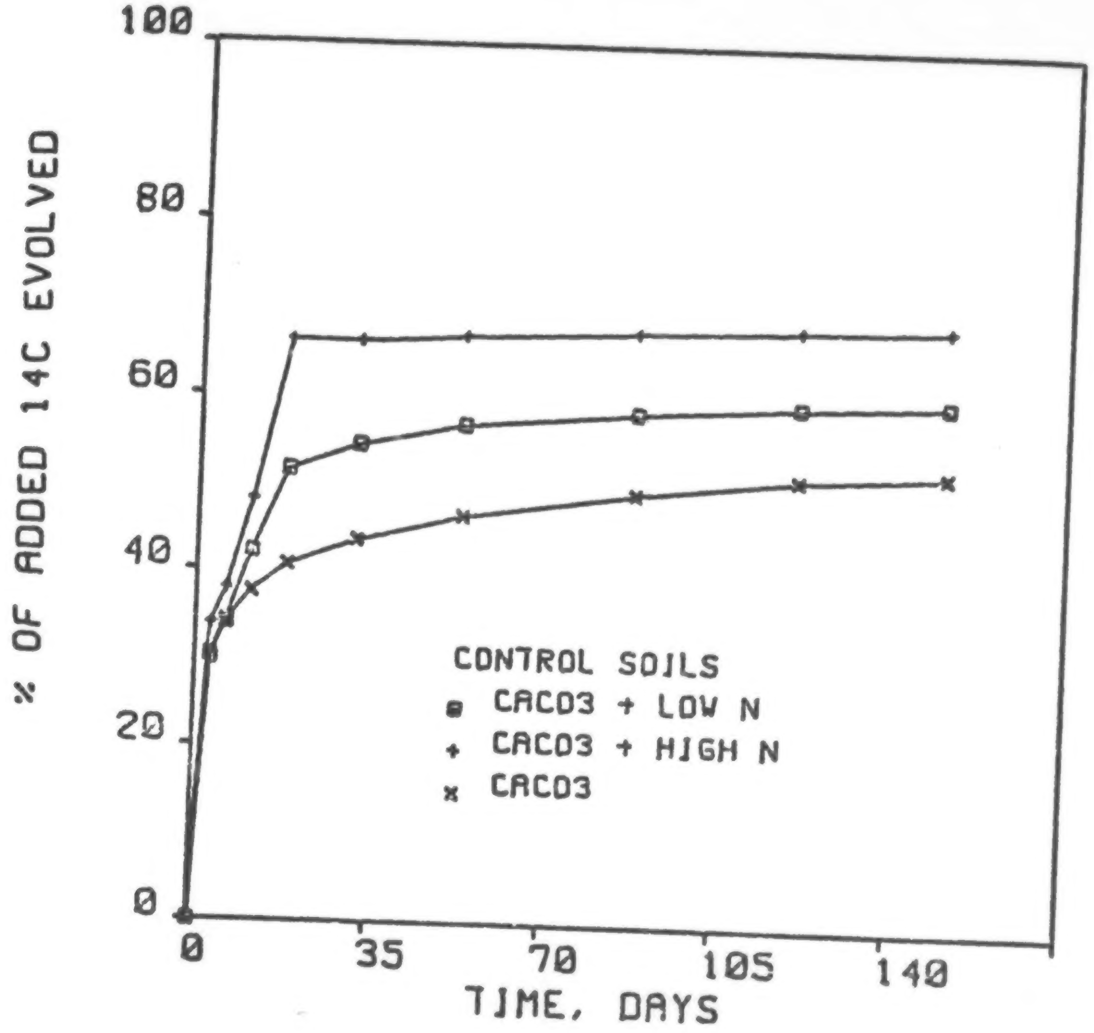

Figure 10.3. Percent of ^{14}C evolved as $^{14}CO_2$ from a Chalmers soil amended with $Ca^{14}CO_3$.

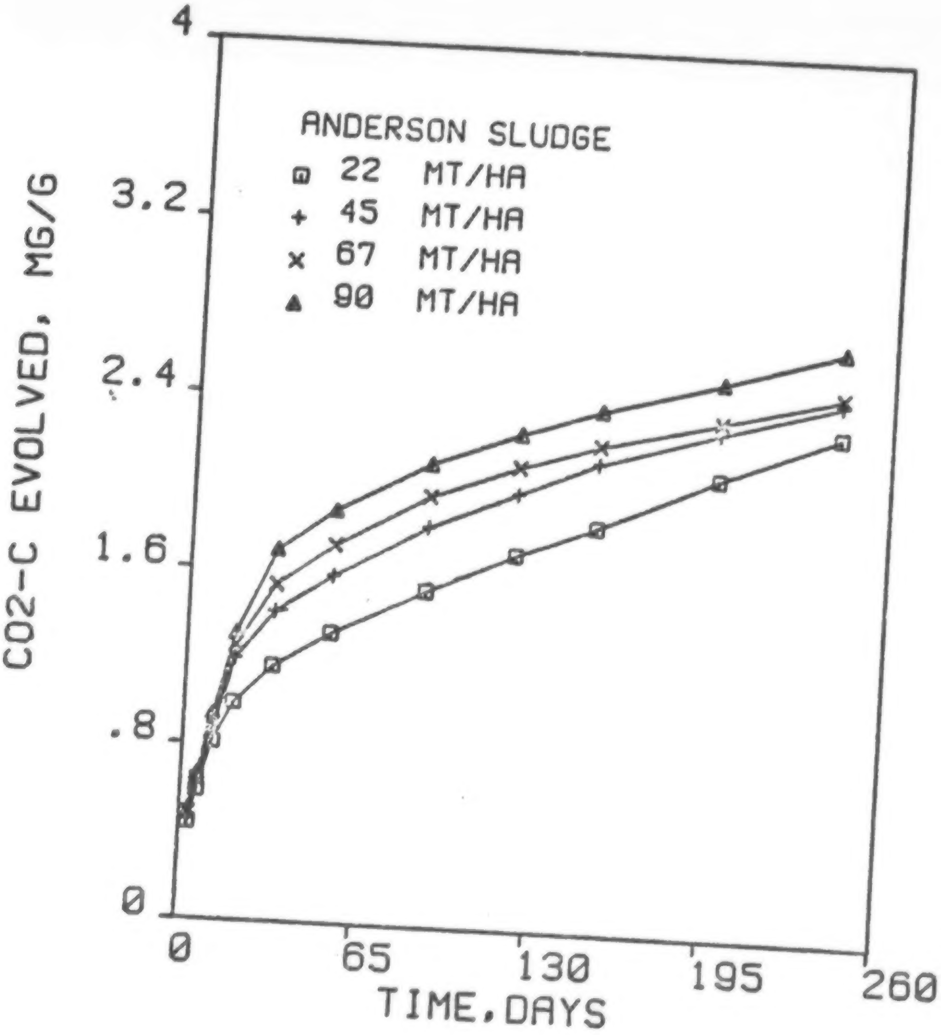

Figure 10.4. Cumulative CO_2-C evolved from a Chalmers sil soil amended with four rates of Anderson sludge.

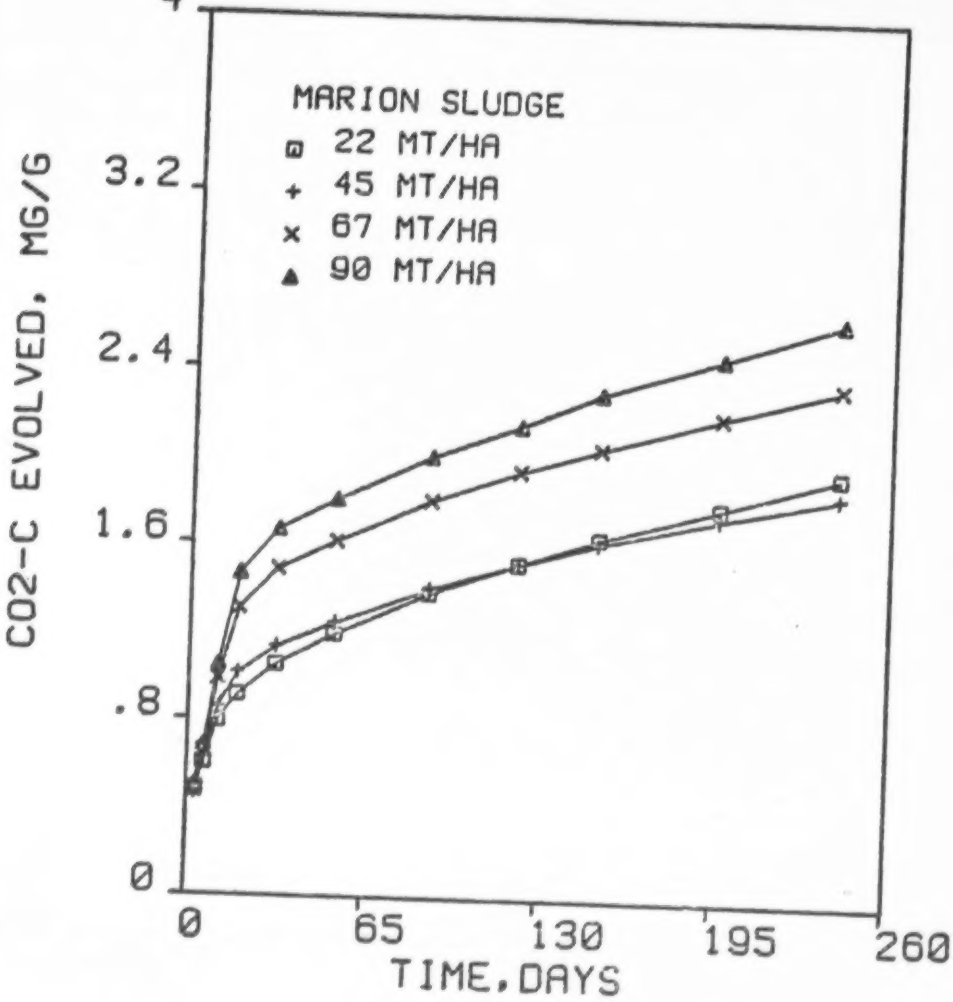

Figure 10.5. Cumulative CO_2-C evolved from a Chalmers sil soil amended with four rates of Marion sludge.

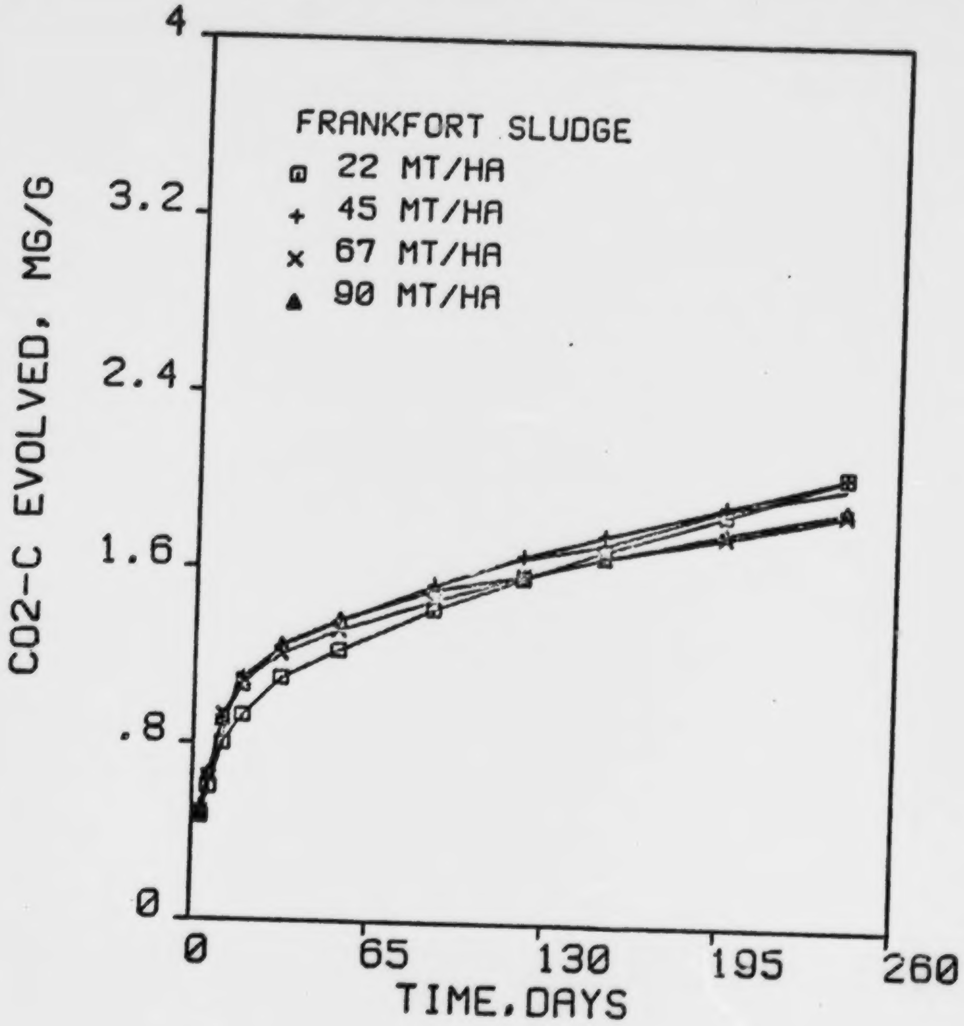

Figure 10.6. Cumulative CO_2-C evolved from a Chalmers sil soil amended with four rates of Frankfort sludge.

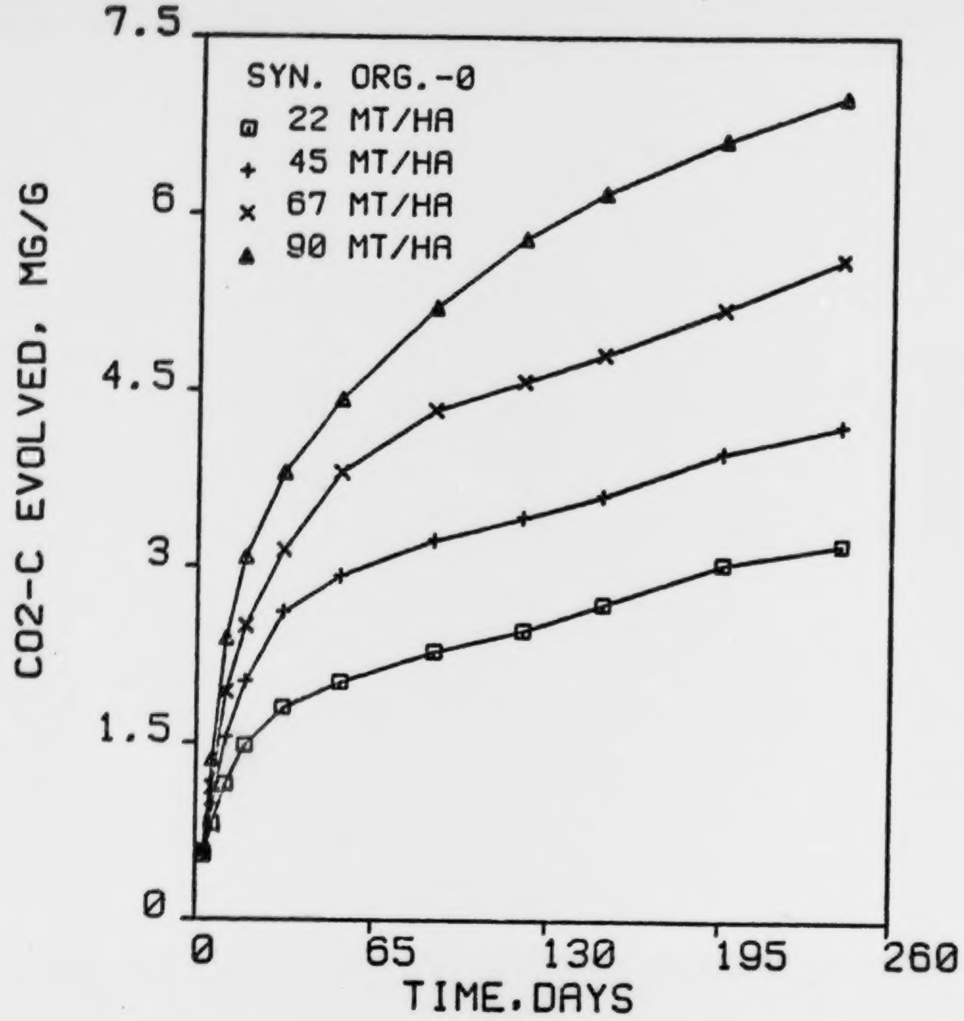

Figure 10.7. Cumulative CO_2-C evolved from a Chalmers sil soil amended with four rates of synthetic sludge.

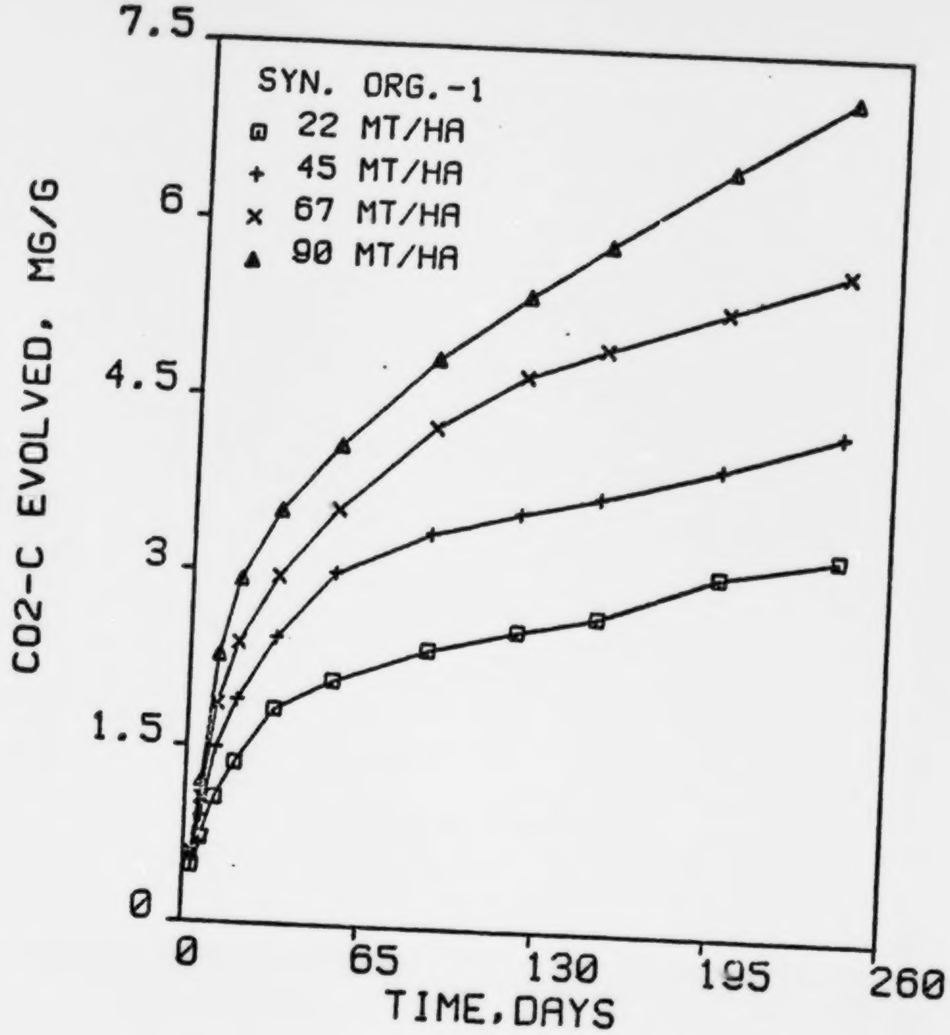

Figure 10.8. Cumulative CO_2-C evolved from a Chalmers sil soil amended with four rates of synthetic sludge.

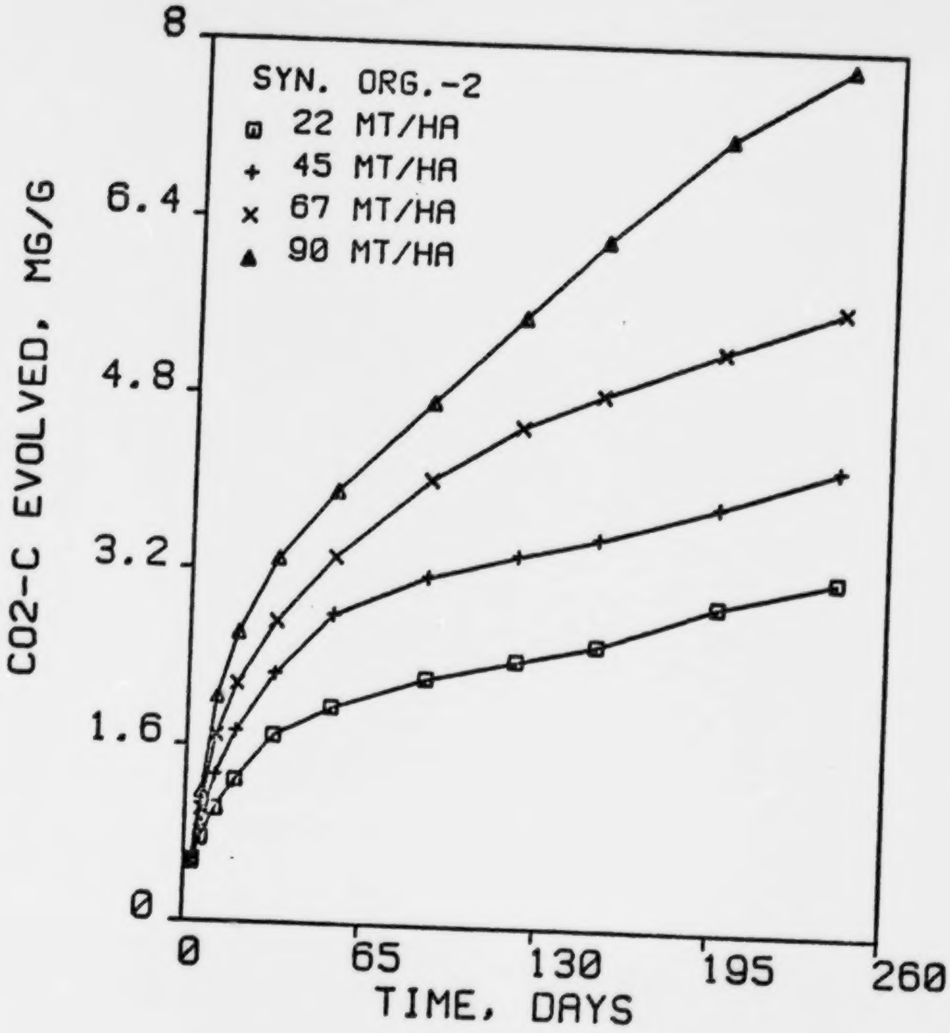

Figure 10.9. Cumulative CO_2-C evolved from a Chalmers sil soil amended with four rates of synthetic sludge.

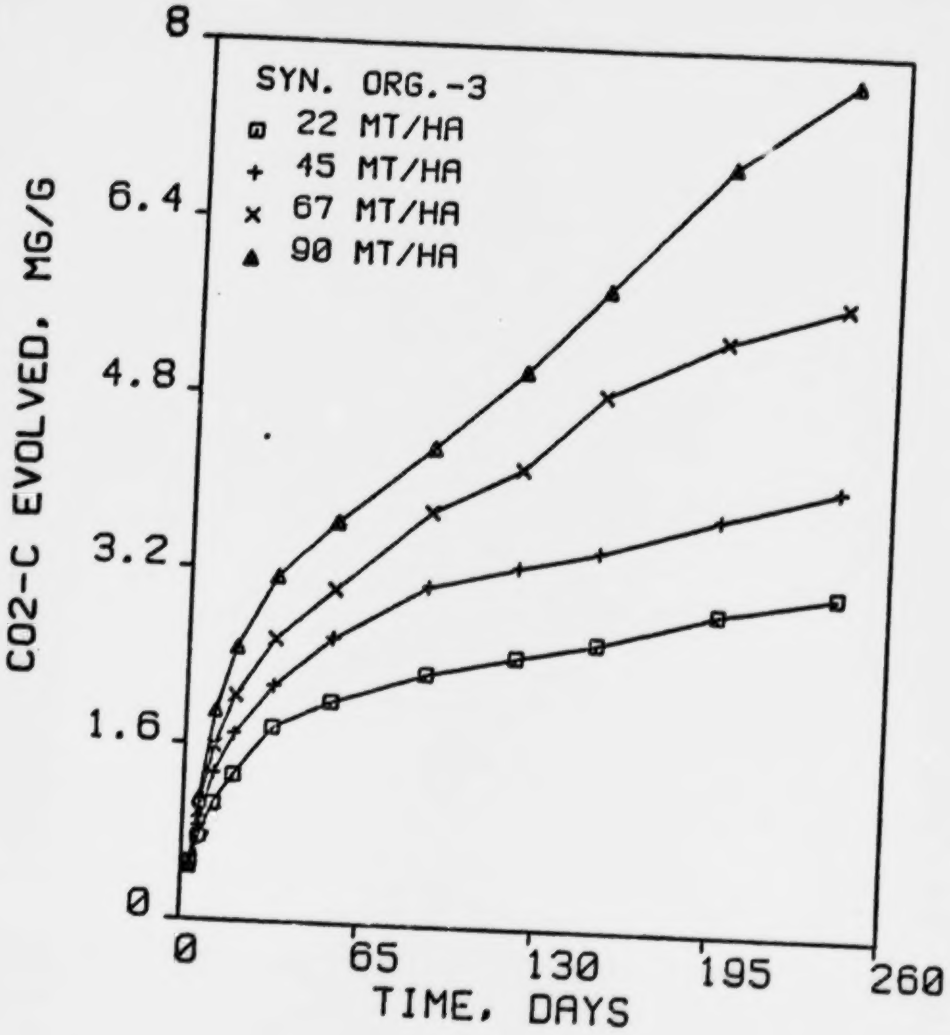

Figure 10.10. Cumulative CO_2-C evolved from a Chalmers sil soil amended with four rates of synthetic sludge.

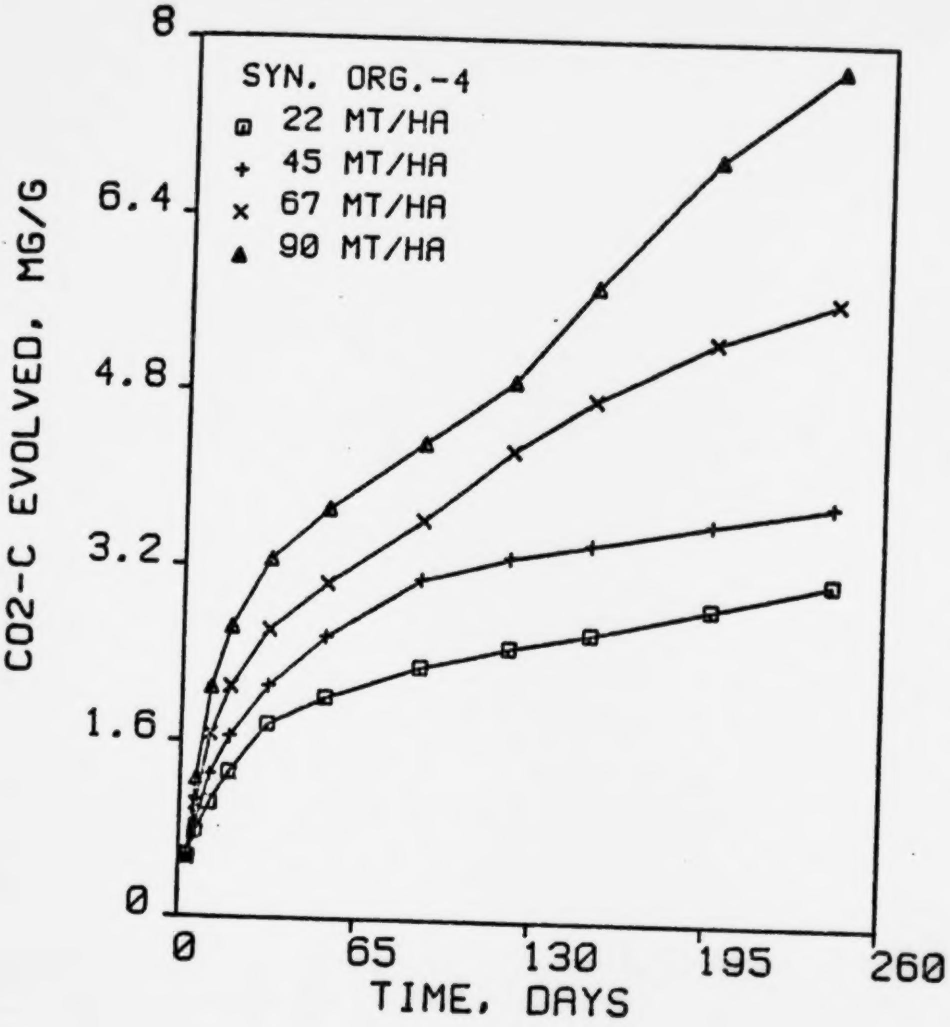

Figure 10.11. Cumulative CO_2-C evolved from a Chalmers sil soil amended with four rates of synthetic sludge.

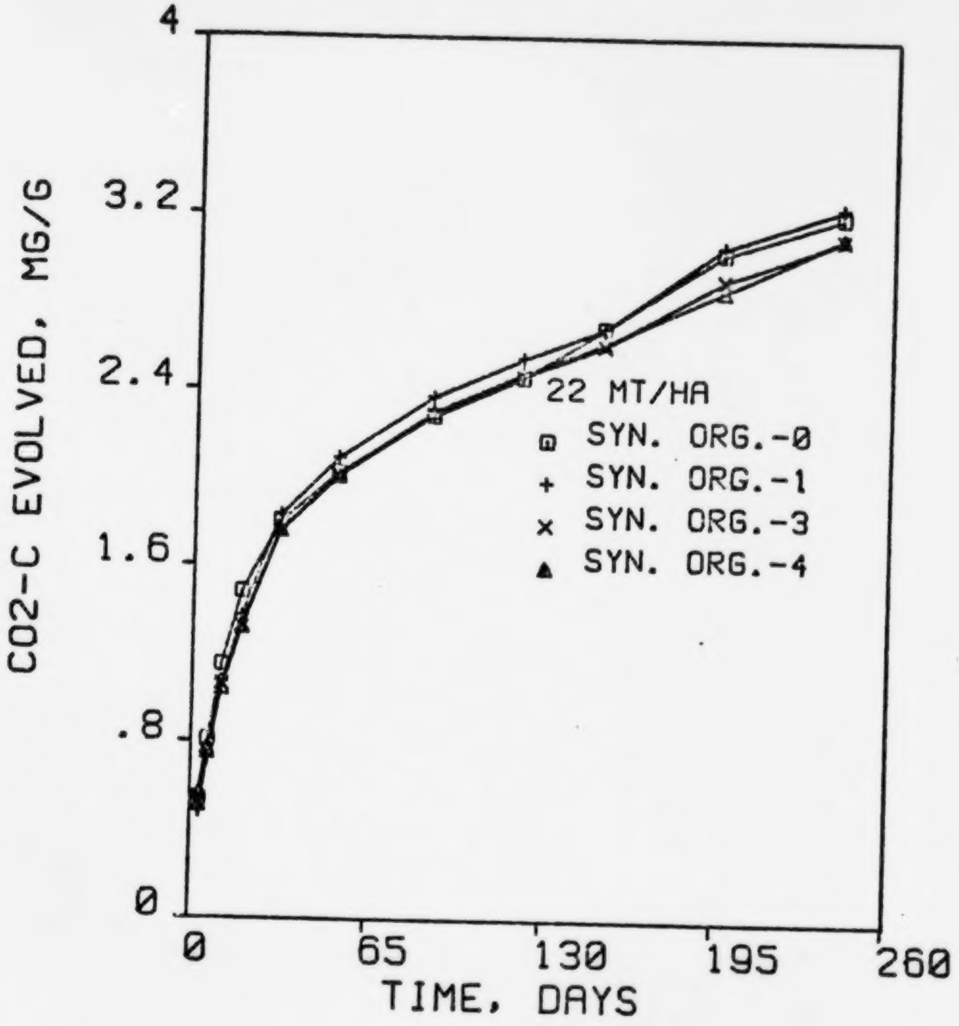

Figure 10.12. Cumulative CO_2-C evolved from a Chalmers sil soil amended with four synthetic organic sludges.

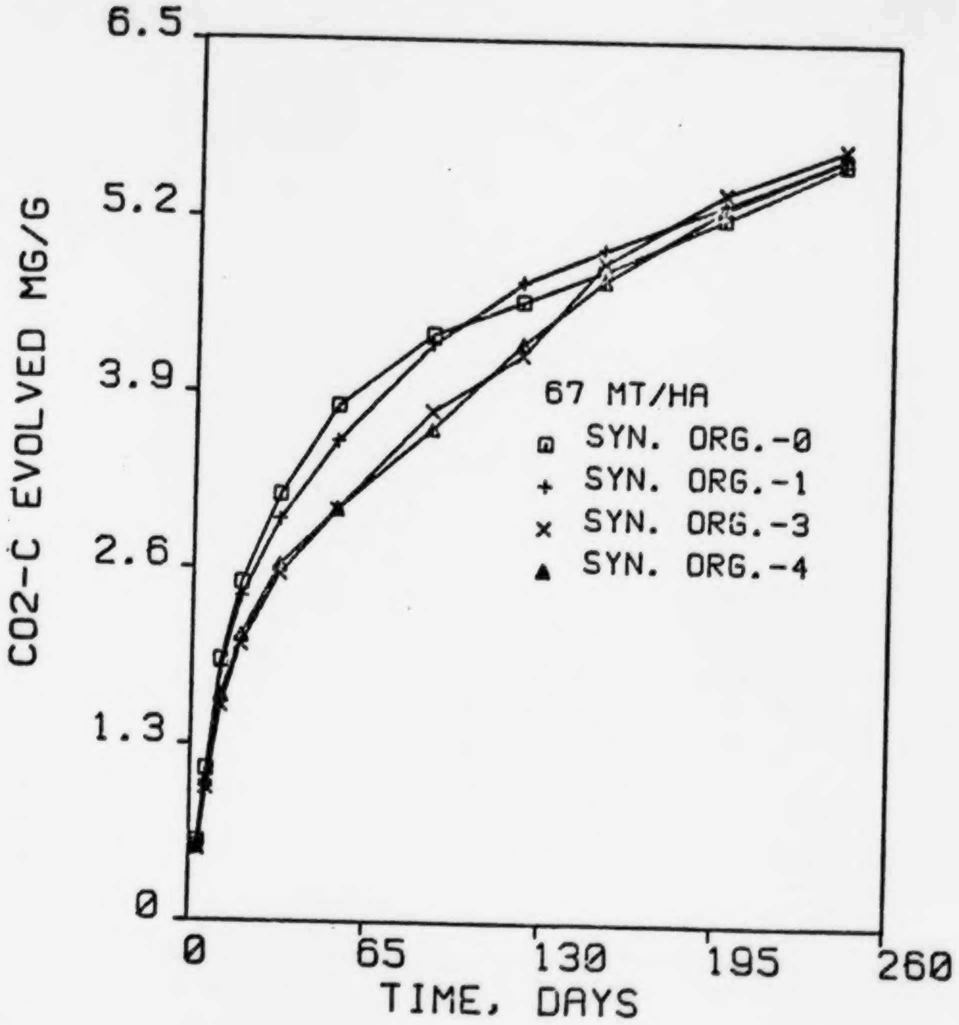

Figure 10.13. Cumulative CO_2-C evolved from a Chalmers sil soil amended with four synthetic organic sludges.

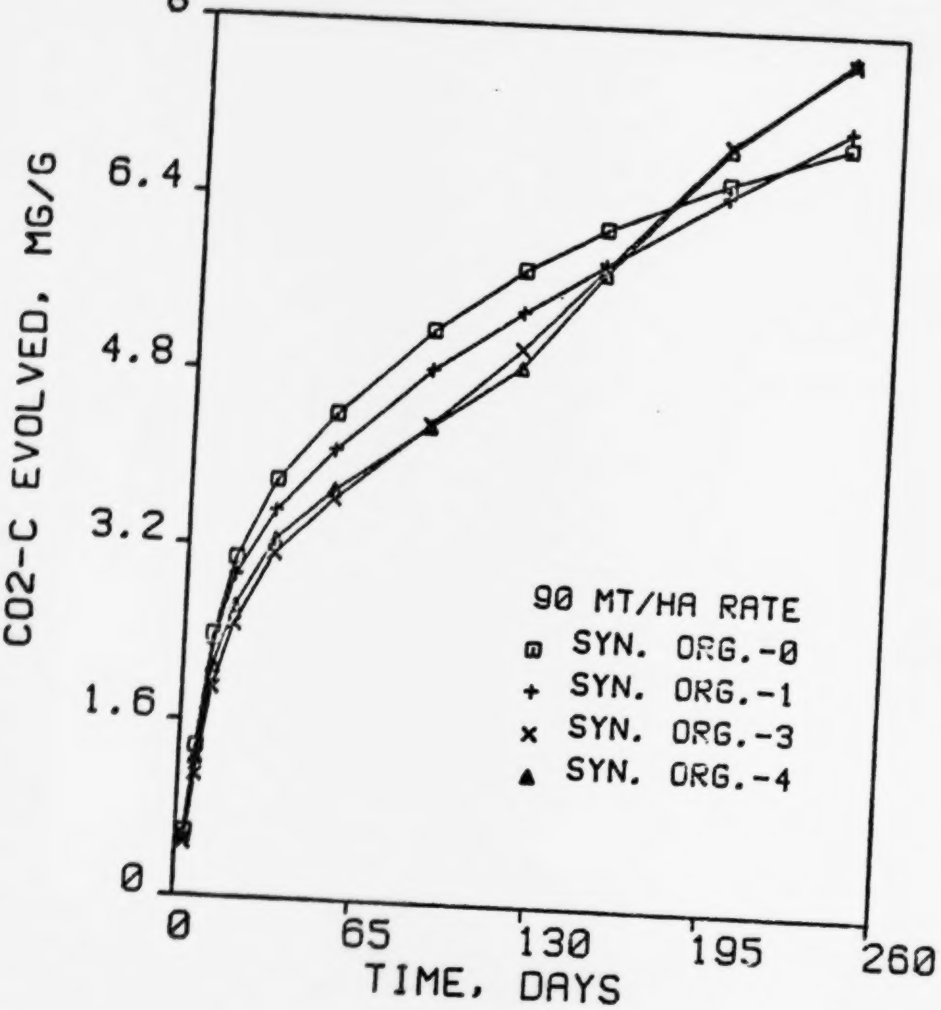

Figure 10.14. Cumulative CO_2-C evolved from a Chalmers sil soil amended with four synthetic organic sludges.

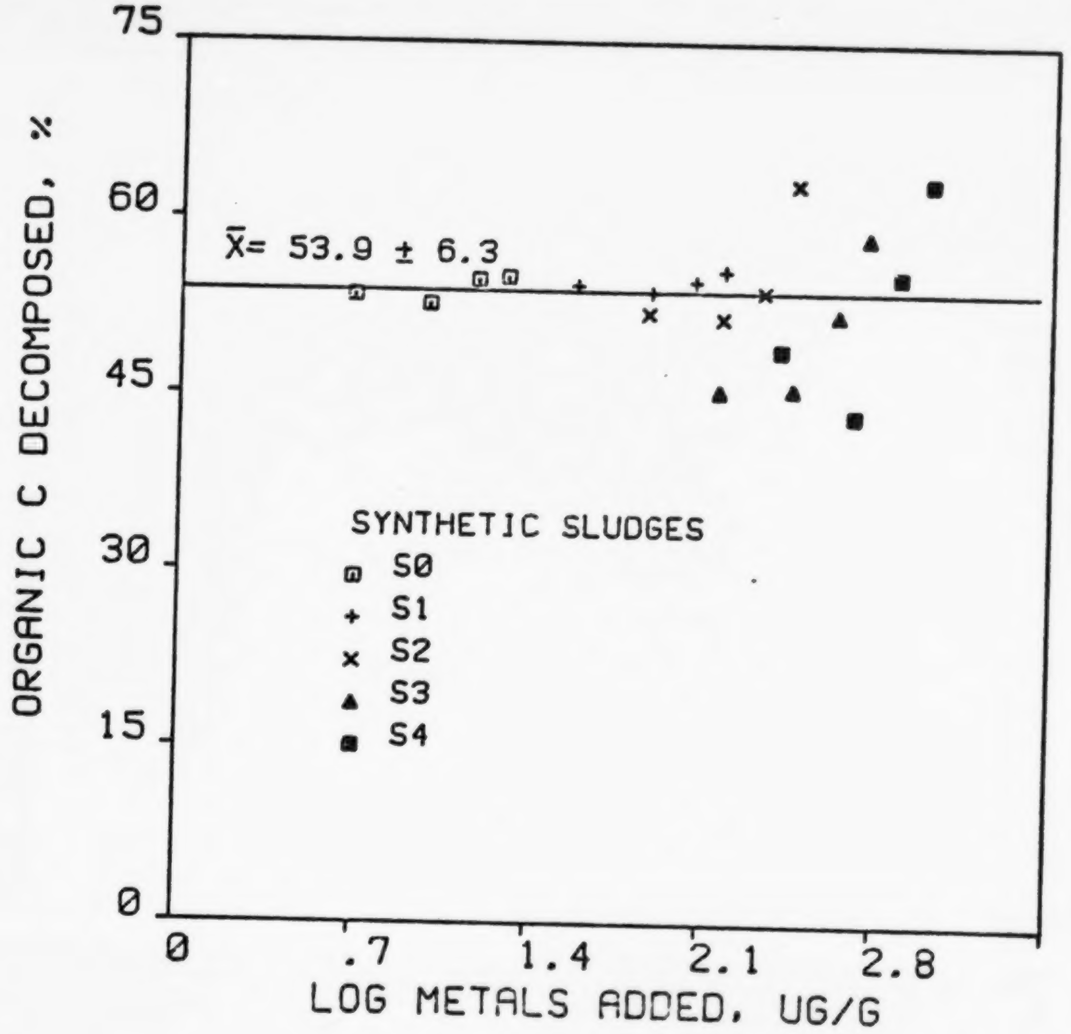

Figure 10.15. Percent of sludge organic C decomposed in a Chalmers sil soil amended with five synthetic sludges of varying heavy metal concentrations. (Log (Zn + Cu + Cd + Ni + Pb) added to soil, μg/g). Horizontal line signifies mean ± standard deviation.

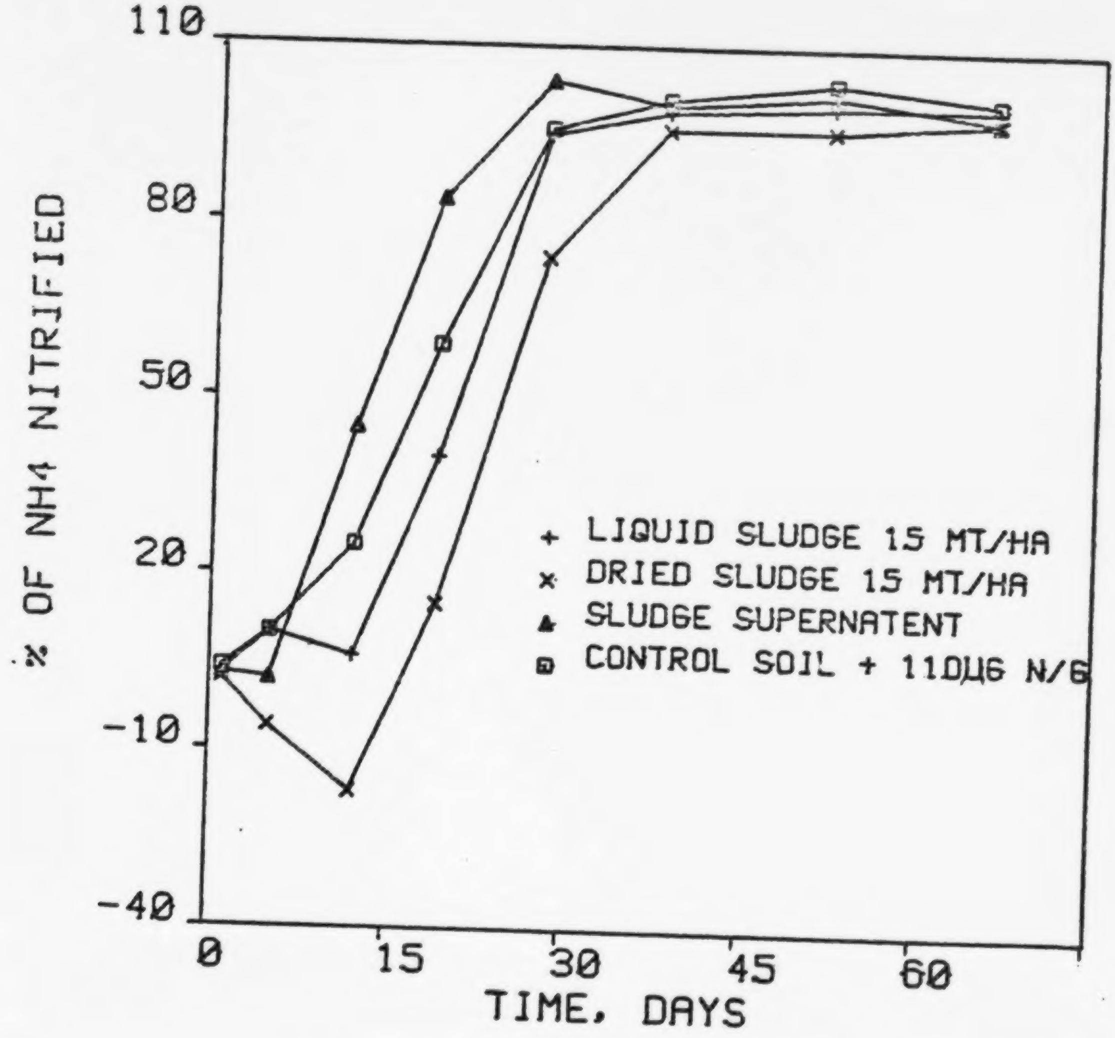

Figure 10.16. Nitrification of NH_4^+ in a Chalmers soil amended with liquid or dried sludge, sludge supernatant, or $(NH_4)_2SO_4$ and incubated for 66 days.

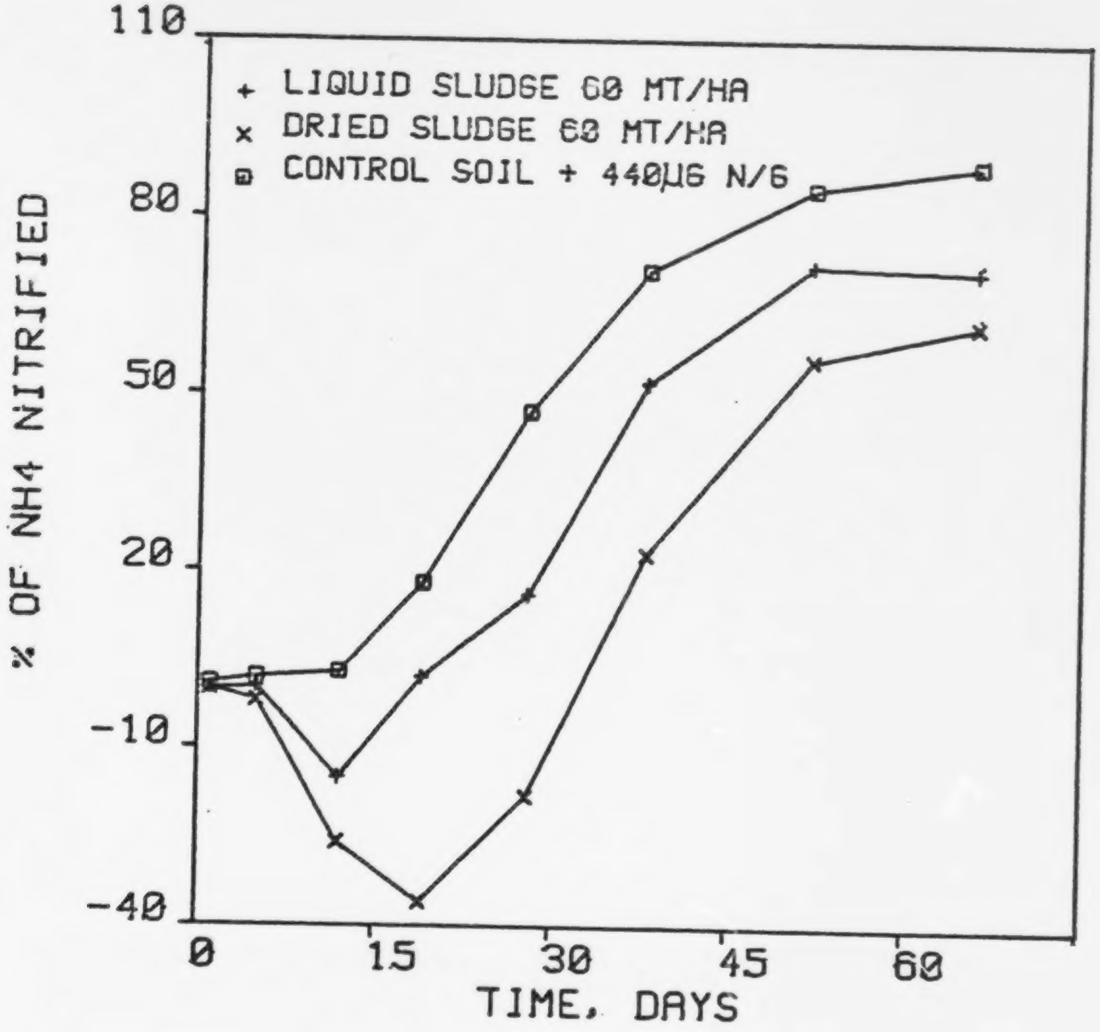

Figure 10.17. Nitrification of NH_4^+ in a Chalmers soil amended with liquid or dried sludge, or $(NH_4)_2SO_4$ and incubated for 66 days.

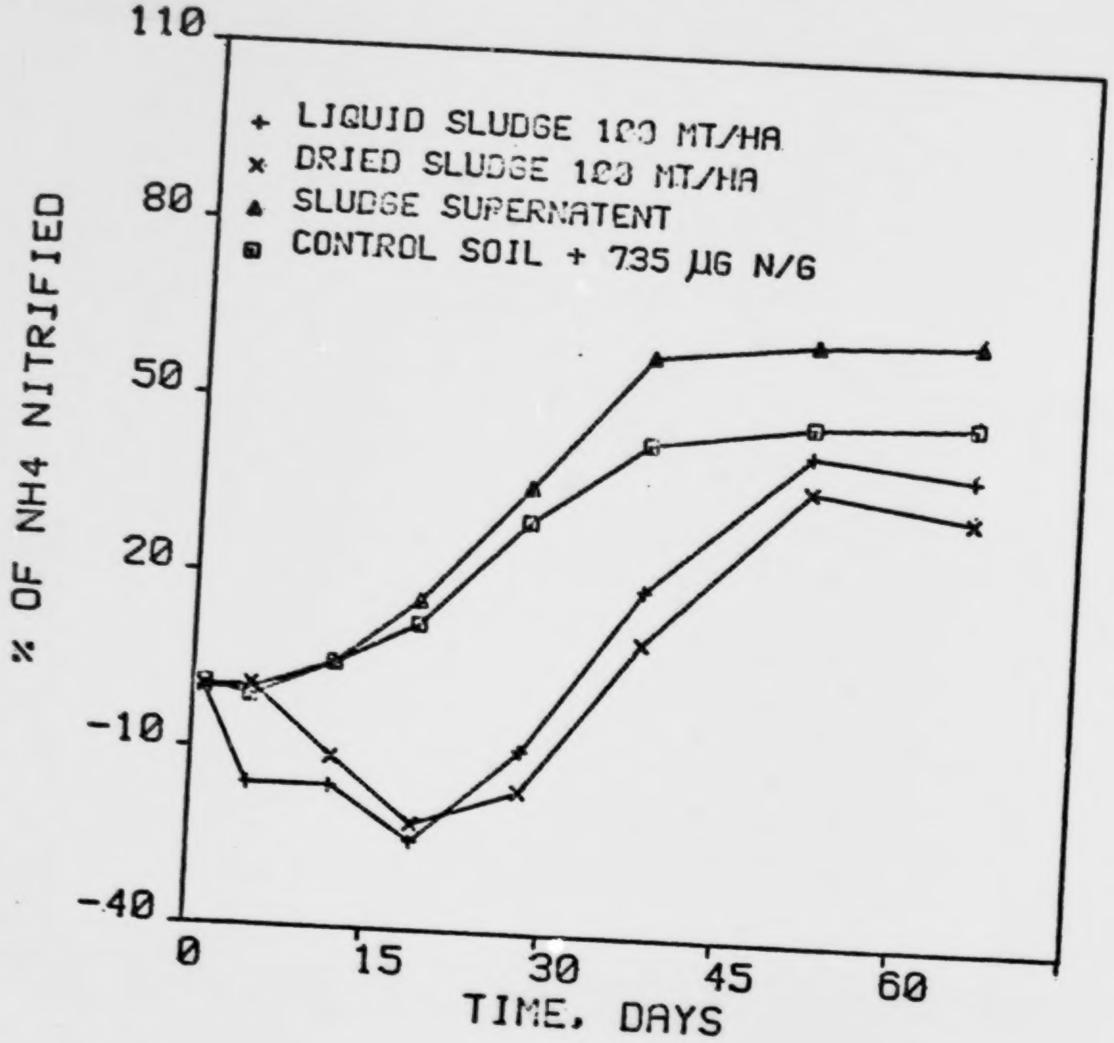

Figure 10.18. Nitrification of NH_4^+ in a Chalmers soil amended with liquid or dried sludge, sludge amendment or $(NH_4)_2SO_4$ and incubated for 66 days.

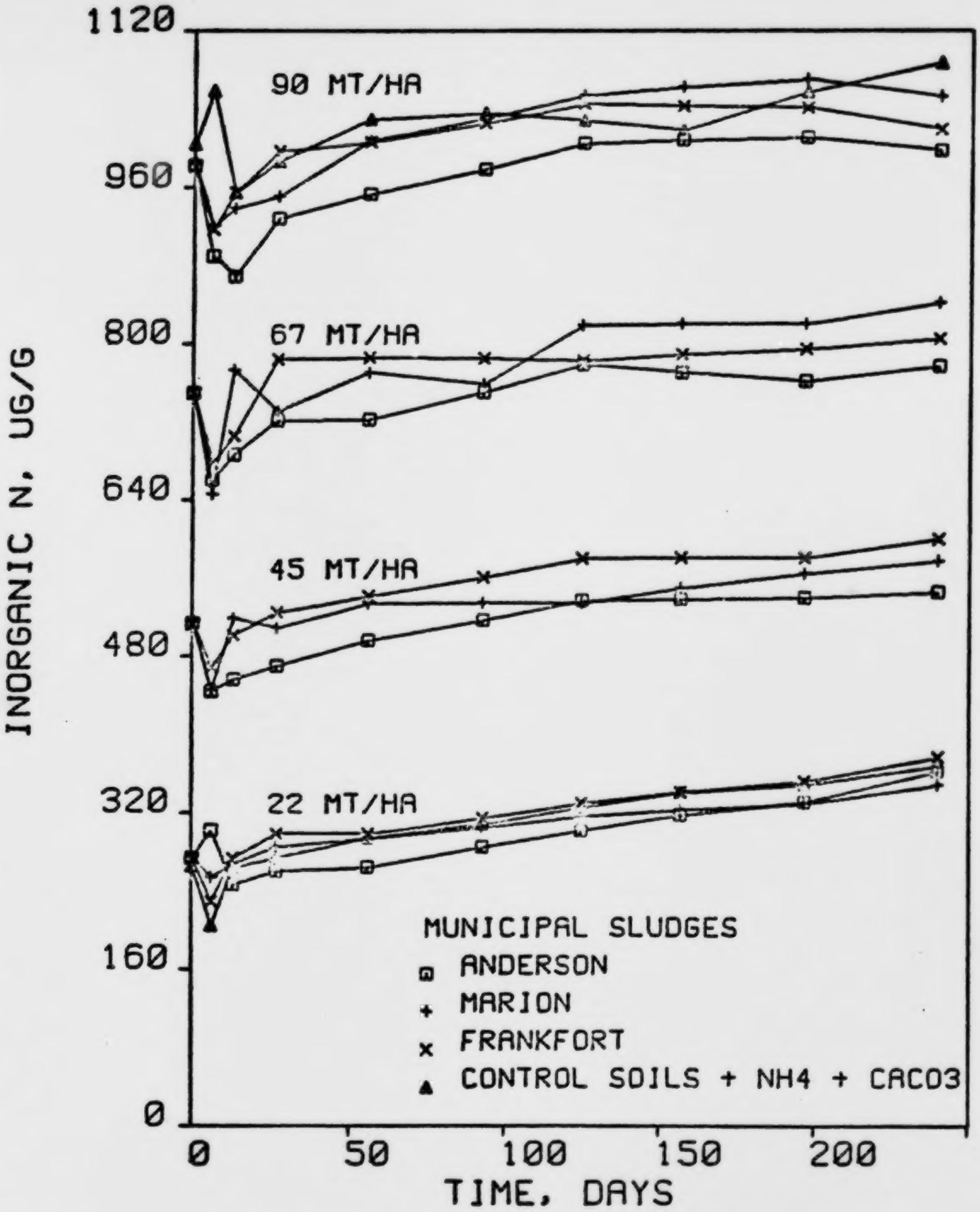

Figure 10.19. Inorganic N concentrations of a Chalmers soil amended with $(NH_4)_2SO_4$ and $CaCO_3$ or municipal sludges and incubated for 66 days.

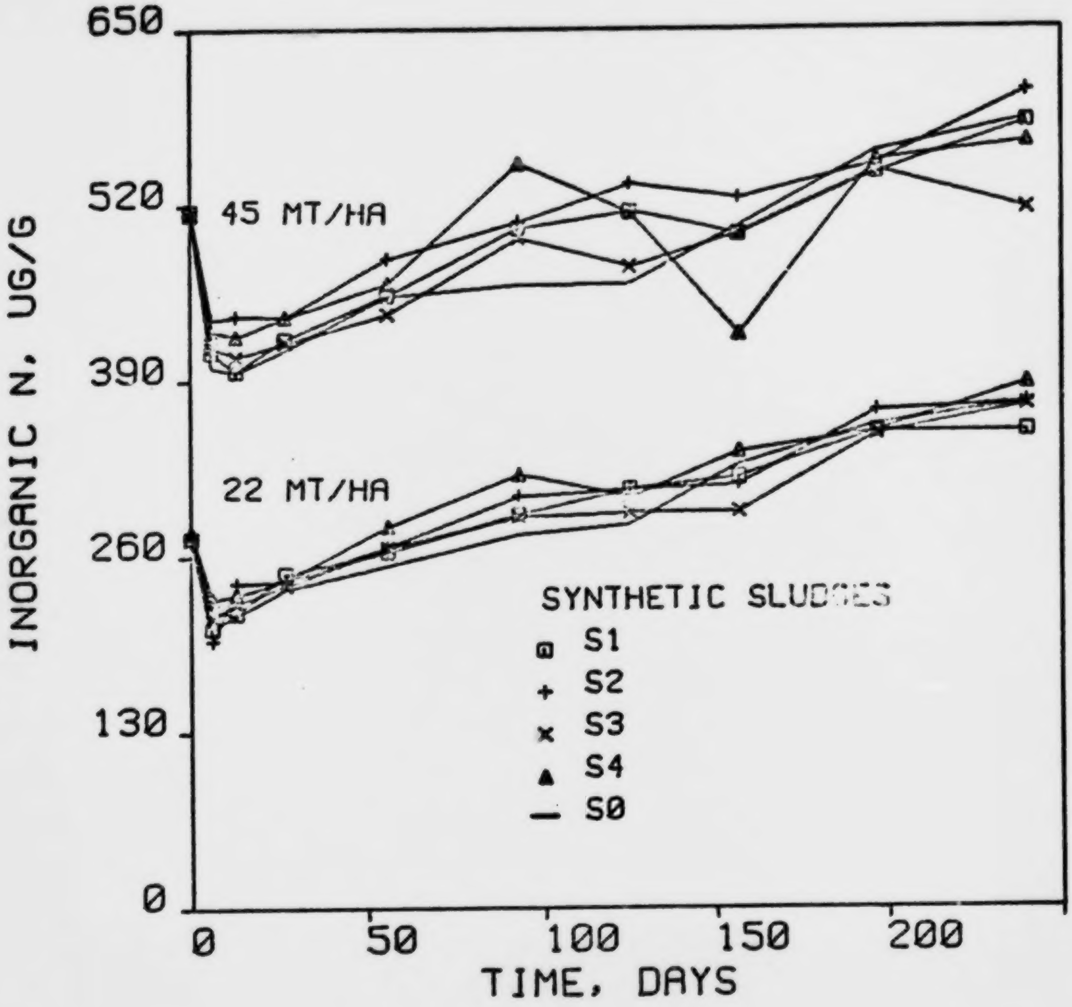

Figure 10.20. Inorganic N concentrations of a Chalmers soil amended with synthetic sludges of varying heavy metal concentrations and incubated for 240 days.

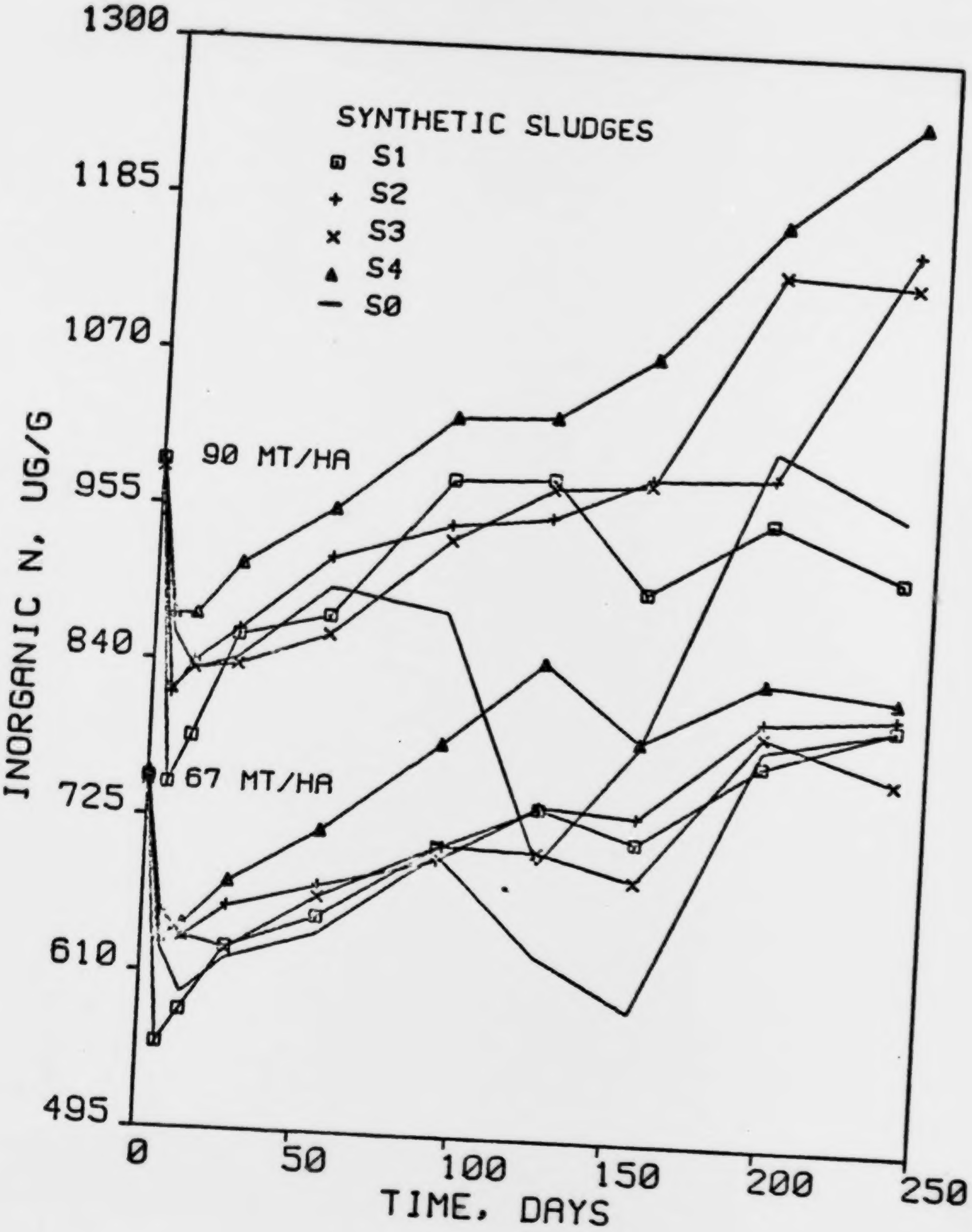

Figure 10.21. Inorganic N concentrations of a Chalmers soil amended with synthetic sludges of varying heavy metal concentrations and incubated for 240 days.

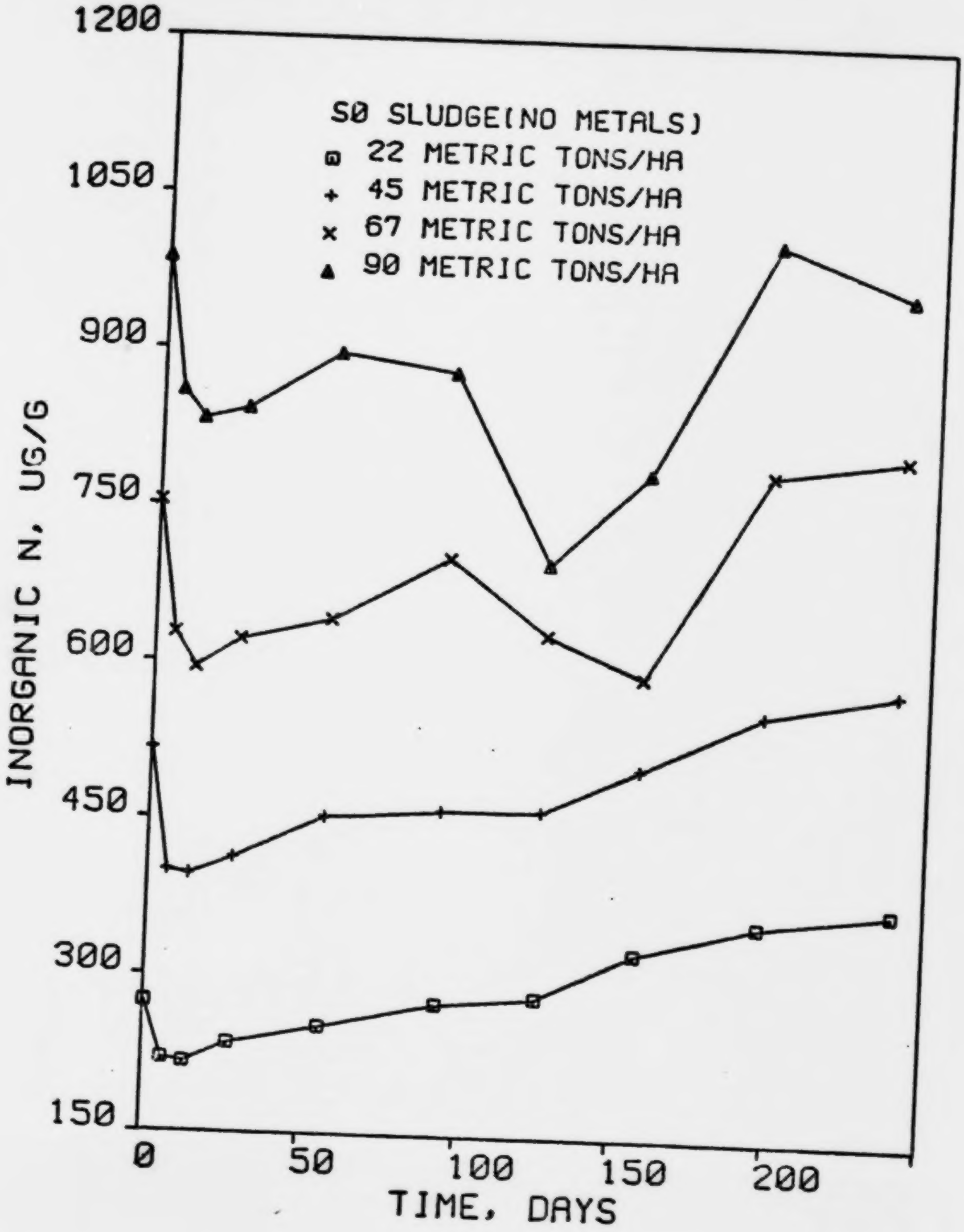

Figure 10.22. Inorganic N concentrations of a Chalmers soil amended with a synthetic sludge containing no added heavy metals and incubated for 240 days.

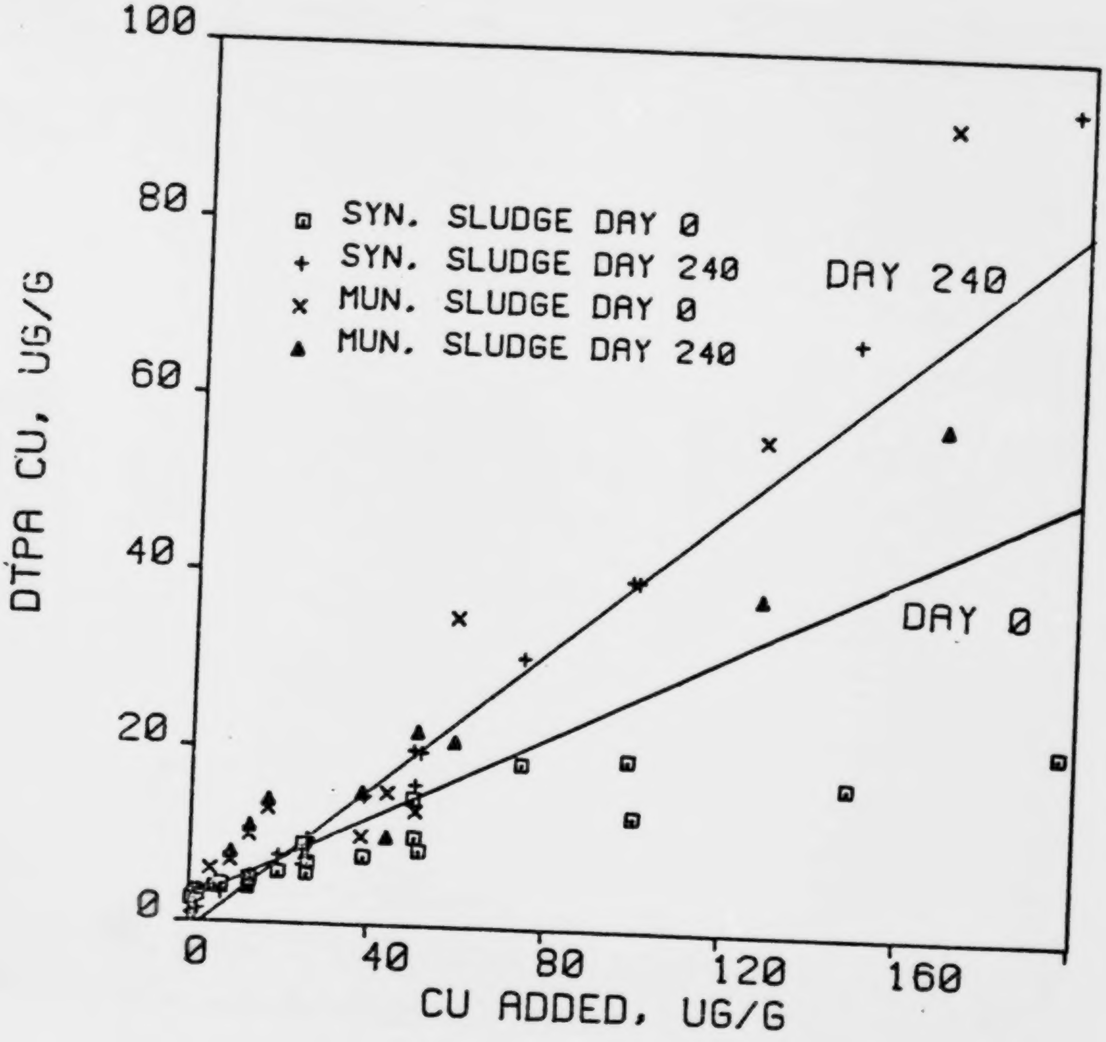

Figure 10.23. Relationship between added and DTPA extractable Cu in a Chalmers soil amended with synthetic and municipal sludges and incubated for 240 days.

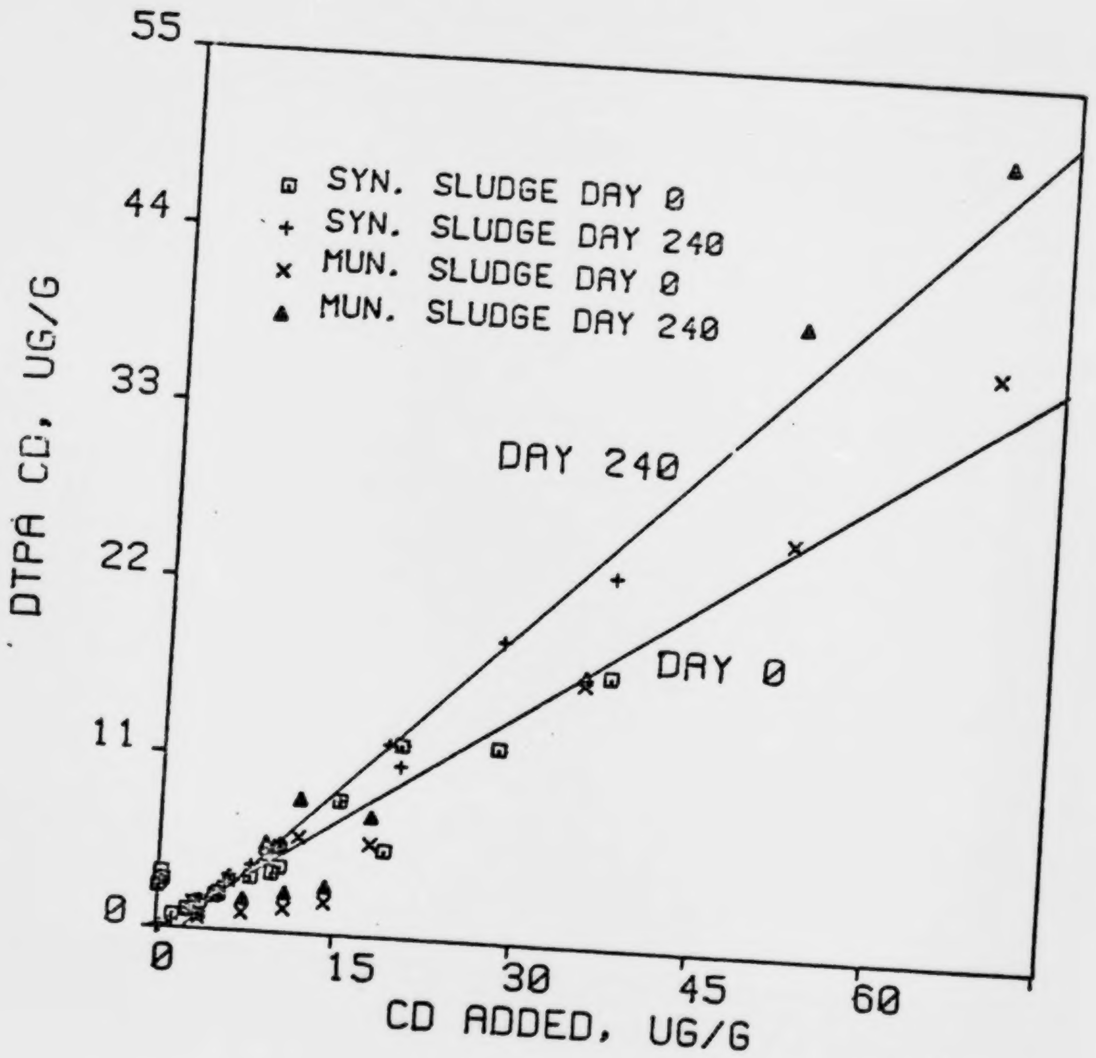

Figure 10.24. Relationship between added and DTPA extractable Cd in a Chalmers soil amended with synthetic and municipal sludges and incubated for 240 days.

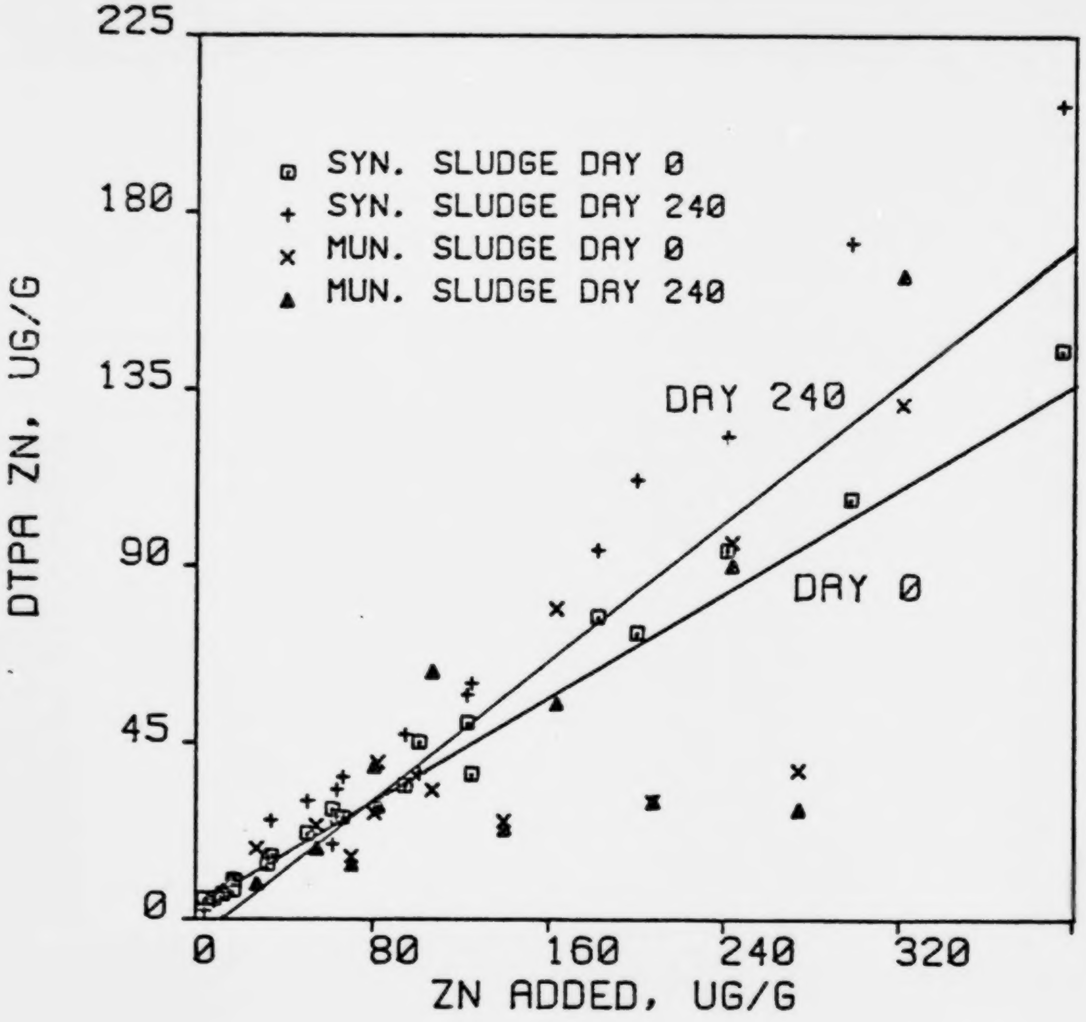

Figure 10.25. Relationship between added and DTPA extractable Zn in a Chalmers soil amended with synthetic and municipal sludges and incubated for 240 days.

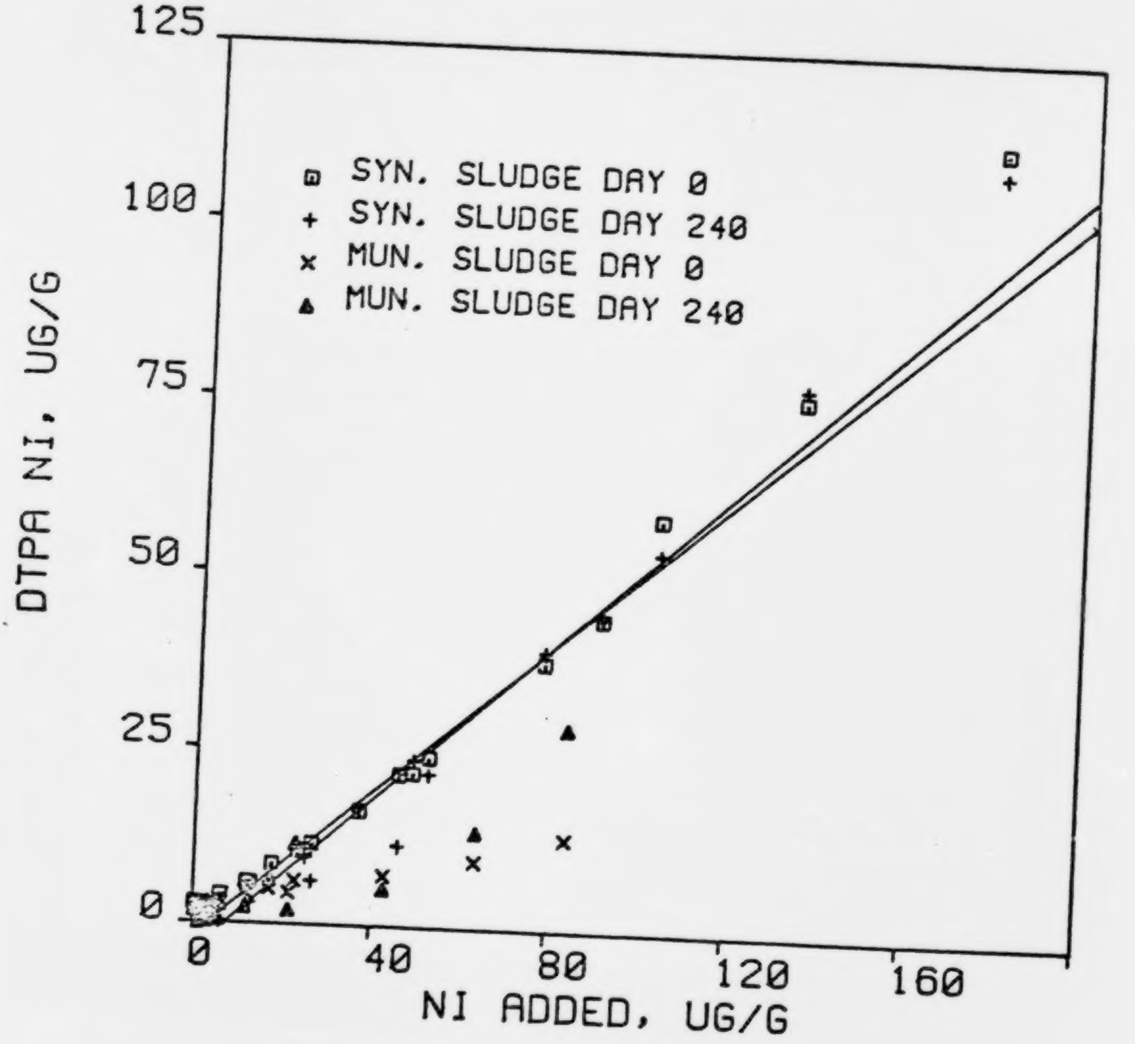

Figure 10.26. Relationship between added and DTPA extractable Ni in a Chalmers soil amended with synthetic and municipal sludges and incubated for 240 days.

SECTION 11

DECOMPOSITION OF SEWAGE SLUDGE IN SOILS UNDER FIELD CONDITIONS

INTRODUCTION

The decomposition of organic C with the resultant evolution of CO_2 is an important microbially mediated process occurring after application of sewage sludge to soils. The extent of organic C decomposition is an important factor in evaluating the sludge-induced positive effects on soil physical properties such as increased tilth, water holding capacity and aggregate stability. Since metals contained in sludges are associated with the decomposable organic fraction (Stover et al., 1977), metal availability to plants could be influenced by the rate and extent of decomposition.

Decomposition of sewage sludge has been evaluated most extensively by laboratory incubation studies. From 18 to 20% of the organic C in an anaerobically digested sludge added to soil was decomposed during six months (Miller, 1974). Terry et al., (1979) found that 26 to 42% of the organic C in anaerobically digested sludge was released as CO_2-C in a 130 day laboratory incubation. Using a short-term incubation to evaluate decomposition of a digested and composted sludge, Tester et al. (1977) found that 16% of the added organic C was evolved as CO_2-C in 54 days and that CO_2 production was linearally related to the rate of compost addition to soils. Hsieh et al., (1981) found that the amount of organic C mineralized for activated and digested sludges over a 6 week period was 26 and 8%, respectively. A one year laboratory study indicated that sludge rather than soil properties (i.e., pH, texture) influence the amount of sludge decomposition (Sommers et al., 1979). Even though total CO_2 evolution increases, the percentage of sludge organic C evolved as CO_2 from soils decreases with increasing sludge application rates (Agbim et al., 1977).

Although laboratory studies allow evaluation of sludge decomposition under controlled conditions, extrapolation of laboratory data to the highly variable environment existing in field situations can be difficult. By determining organic C in treated and untreated soils, Varanka et al., (1976) found that approximately 50% of the sludge organic C was decomposed within six years after application. Field decomposition studies utilizing measurements of CO_2 evolution have been useful to evaluate the factors influencing decomposition of organic compounds and residues in soils (Edwards, 1975; Wildung et al., 1975; Kowalenko et al., 1978).

Data from both field and laboratory studies have been used to develop models for predicting the decomposition of organic materials in soils as influenced by temperature, moisture, fertilizer additions and chemical composition of the decomposing material (Parnas, 1975; Gilmour and Beck, 1978; Hunt, 1977; Edwards, 1975; Kowalenko et al., 1978; Burnell et al., 1977a and b). Development of such a model for the decomposition of sewage sludge in soil could aid in the understanding of the fate of the sludge materials in soil and in development of guidelines for sludge application rates to land.

Recent work on modeling sludge decomposition in soils has commonly utilized first-order relationships between organic C remaining and time. By evaluating the effect of time, sludge application rate and soil temperature on cumulative organic C mineralized, Hsieh et al., (1981) developed a model consisting of exponential functions. Data from a short-term laboratory incubation with activated and digested sewage sludges were used to test the model developed. By utilizing data from the literature, Reddy et al., (1980) evaluated first-order rate constants for decomposition of plant, animal and organic wastes in soil. Waste decomposition was separated into two or three phases with first-order kinetics being used for each phase. Rapid decomposition of organic C in phases one and two was followed by decomposition of more resistant organic C fractions. Rate constants calculated for each phase of decomposition were corrected for soil temperature, moisture and method of application. These authors reported that first order rate constants for decomposition of sewage sludge in soil at 21°C were 0.0097 and 0.0052 day^{-1} for the rapid and slow phases, respectively. Using a similar approach, Gilmour and Gilmour (1980) developed a simulation model for sludge decomposition in soil using data from Terry et al. (1979a, b). Sludge decomposition was again separated into rapid and slow phases with each first-order rate constant being corrected for soil temperature and moisture. They found that the month of sludge addition to soils had only a small effect on the calculated overall extent of decomposition and that laboratory studies conducted under optimum conditions would overestiamte field decomposition rates when field temperature and moisture diverge from optimum.

In summary, laboratory studies have shown varying degrees of decomposition following application of sludges to soil. The main factors influencing decomposition include sludge composition and temperature and moisture of the soil. Few field studies have been conducted to measure the decomposition of sewage sludges in soils under field conditions. Several workers have measured the effects of time, temperature, moisture and C substrate characteristics on decomposition of organic wastes (e.g., plant residues). Models based on first-order kinetics have been recently proposed to describe decomposition of sludge in soils. The objectives of this study were: (1) to evaluate sludge decomposition under field and laboratory conditions; (2) to compare data obtained on sludge decomposition from field and laboratory studies; (3) to evaluate a model for decomposition of sludge organic C applied to soil; and (4) to determine the effects of prior sludge treatment processes on decomposition of sludge in soil.

MATERIALS AND METHODS

Partially dried sewage sludges used in this field study were obtained from sewage treatment plants at Anderson, Marion, and Frankfort, Indiana, and Chicago, Illinois. A liquid sludge sample was obtained from the anaerobic digester at the West Lafayette sewage treatment plant. A subsample of the West Lafayette sludge was dried at 70°C to compare decomposition of liquid and dried sludge. All dried sludges were ground to pass a 60 mesh screen. Sludge properties are listed in Table 11.1.

The study was conducted at the Purdue Agronomy Farm on a Chalmers silt loam soil (Typic Argiaquoll). The plot area (approximately 3 by 4.5 m) was cleared of vegetation and 35 cm long by 10.2 cm diameter polyvinyl chloride (PVC) cylinders were placed into the soil on August 22 and 23, 1978. The cylinders were arranged in three rows of 10 cylinders per row (10 treatments, 3 replicates) with each of the cylinders approximately 30 cm apart and the rows approximately 90 cm apart. The cylinders were placed in the soil using the following procedure: a hydraulic probe was used to remove the upper 15.2 cm of soil. A PVC pipe was then forced into the cavity until 10.2 cm of the PVC pipe remained above the soil surface. The surface soil samples (upper 15.2 cm) previously removed from all cylinder locations were composited, air-dried, and ground to pass a 8.5 mm screen. The average amount of air-dry soil removed from each cylinder location (1,850 g) was then weighed into paper bags and mixed with the appropriate amount of air-dry sewage sludge. The Anderson sludge was added at rates equivalent to 40, 100, and 200 metric tons/ha (32.1 g/column, 80.3 g/column, and 160 g/column). The West Lafayette sludges (air-dried and liquid) were added at 25 metric tons/ha (20.3 g/column). The Frankfort, Marion and Chicago sludges were added at the 100 metric tons/ha rate. Since some sludges contain substantial amounts of $CaCO_3$ which will contribute to total CO_2 evolution, triplicate soil columns were treated with $CaCO_3$-C equivalent to the inorganic C added in the Marion sludge (4.97 g $CaCO_3$-C/column). The soil-sludge mixtures were then placed in the PVC cylinders and water was added to bring the soil to ~ -0.6 bar tension (.215 g H_2O/g soil) (determined on a pressure plate apparatus). To uniformly wet the soil-sludge mixtures, one-fourth portions of sludge-soil mixture and H_2O were added sequentially to the PVC cylinders. The 25 metric tons/ha rate of the liquid West Lafayette sludge contained sufficient water to bring the soil-sludge mixture to ~ -0.6 bars water tension. The soils were allowed to equilibrate in the cylinders for 24 hours before initial measurements of CO_2 evolution.

Evolution of CO_2 was measured over a 24 hour period and involved placing vials containing either 20 or 25 ml of 2 $\underline{M}$ KOH inside the cylinders approximately 3 cm above the soil surface (metal clips held the vials above the soil surface). Aluminum foil covered plexiglas lids with rubber gaskets were used to seal the top of the cylinders. After 24 hours, the vials were removed and the CO_2 evolved was determined by adding excess 1.5 $\underline{M}$ $BaCl_2$ to 5 ml aliquots of KOH and titrating to the phenolphthalein end point with standard HCl. To correct for CO_2 in the head space of the cylinders, PVC cylinders 10.2 cm in height with sealed bottoms were placed in the field and CO_2 was collected and analyzed as described above. Soil moisture was measured gravimetrically by removing 2.5 cm diameter x 15.2 cm soil cores adjacent to the cylinders and oven-drying subsamples at 105°C for 16 hours. The moisture values (percent H_2O) of the field soil were converted to water tension values (-bars) utilizing a soil moisture (percent water) - water tension (-bars) curve. The curve was developed from pressure plate measurements of samples of the field soil at different moisture levels. The curve describing the relation between the percent H_2O content in the soil and soil H_2O tension fit the equation $y = 16.99 - 7.68 \ln X$ ($r^2 = .96$) where y = percent H_2O in soil, and x = H_2O tension (-bars). In addition, soil temperature and rainfall data were obtained from the weather station at the Purdue Agronomy Farm.

Analysis of the soil or soil-sludge mixture after the end of the experimental period were as follows: organic carbon as described by Mebius (1960) using N-phenylanthranillic acid as the indicator; inorganic C as described by Bundy and Bremner (1972); pH of soil mixtures was measured in 1:1 soil:distilled water with a glass electrode.

Calculations

The total CO_2-C evolved from the cylinders at the end of 423 days was calculated from the amount of CO_2-C evolved per day, assuming that the rate of CO_2 evolution on the sampling date represented an average value for the number of days elapsed between the midway points on either side of the sampling date. This 'average' rate of CO_2 evolution was multiplied by the number of days in the sampling period to calculate the amount of CO_2 evolved (mg C/cylinder). These values were then summed to obtain the total cumulative CO_2-C evolved.

The amount of added sludge organic C decomposed in the experimental period can be calculated from either CO_2-C evolved or direct measurement of organic C in the soil at the end of the study. In all cases, the calculated value of org. C decomposed is divided by the organic C added to obtain percent organic C decomposed. Using data from CO_2-C evolution measurements, the simplest approach involves correcting each treatment for the control. That is,

$$\text{Org. C decomposed} = (CO_2\text{-}C_{\text{trtmt.}} - CO_2\text{-}C_{\text{control}}) \; /$$

However, since some of the sludges contain considerable inorganic C (primarily Ca and Mg carbonates), initial soil acidity (pH 5.2) plus H^+ liberated by nitrification of NH_4^+ would result in partial dissolution of the carbonates added. Therefore, CO_2 resulting from decomposition of sludge organic C was also evaluated by correcting the CO_2 evolved for the soil control (no sludge added) and for a soil control treated with $CaCO_3$. The calculations were

$$\text{Org. C decomposed} = (CO_2\text{-}C_{\text{trtmt.}} - CO_2\text{-}C_{\text{control}} - CO_2\text{-}C_{CaCO_3})$$

where

$$CO_2\text{-}C_{CaCO_3} = \frac{(CO_2\text{-}C_{\text{control} + CaCO_3}) - (CO_2\text{-}C_{\text{control}})}{CaCO_3\text{-C added to control}} \times CaCO_3 \text{ in sludge}$$

The above calculations proportion the correction for CO_2-C from inorganic C dissolution in each sludge treatment based on the $CaCO_3$ control and the amount of inorganic C initially present in each sludge. This calculation assumes that the rate and extent of $CaCO_3$ dissolution in the soils amended with reagent grade $CaCO_3$ and sludge-borne $CaCO_3$ were identical.

Alternatively, the cumulative amount of CO_2-C evolved may be corrected for inorganic C dissolution by direct determination of the inorganic C present in the soil-sludge mixture after the experimental period (all units are mg C/column).

$$\text{Org. C decomposed} = [(CO_2\text{-}C_{\text{trtmt.}} - CO_2\text{-}C_{\text{control}}) - (\text{Initial inorg. C} - \text{Final inorg. C})]$$

Soil analysis for organic C remaining after the incubation period was also used to estimate sludge organic C decomposition:

$$\text{Org. C decomposed} = \%\ \text{Org. } C_{\text{trtmt.}} - \%\ \text{Org. } C_{\text{control}}$$

The majority of the results are averages of duplicate determinations on triplicate experimental systems.

RESULTS AND DISCUSSION

Sludge Composition

The sewage sludges used in the field study had been anaerobically digested and were aged on a drying bed or equivalent at sewage treatment plants in Anderson, Marion and Frankfort, Indiana and Chicago, Illinois. A liquid sludge sample was obtained from the anaerobic digester at the West Lafayette, Indiana, sewage treatment plant. A subsample of the West Lafayette sludge was dewatered by filtration and then dried at 70°C in order to compare decomposition of liquid and dried sludge. Sludge properties are shown in Table 11.1. The organic C content of the dried sludges ranged from 7.5 to 32% with an average of 16.4%. Dewatering a liquid sludge (West Lafayette) under laboratory conditions resulted in losses of organic C during either filtration or drying at 70°C. It is noted, however, that dewatering the liquid sludge did not reduce the organic C or volatile solids to the extent of that observed for the other dried municipal sludges. Inorganic C content of the sludges ranged from 0 to 2% except for the Marion sludge which had been lime treated (5.97%). The inorganic and organic N levels for the dried sludges ranged from 0.01 to 0.14% and 0.50 to 3.29%, respectively. Although dewatering the liquid West Lafayette sludge under laboratory conditions reduced the inorganic N to a level similar to that found in the other dried municipal sludges, the organic N level was not markedly reduced.

Fate of Inorganic C

The inorganic C (primarily $CaCO_3$) added to the soil, especially in those sludges receiving a lime treatment, can significantly affect organic C decomposition values based on CO_2-C evolution. Due to the low initial pH (5.2) of the Chalmers soil and H^+ liberated by NH_4^+ during nitrification, partial dissolution of added inorganic C occurred after sludge application.

As shown in Table 11.2, all sludge, except the West Lafayette, increased soil pH above 5.2. The control plus $CaCO_3$, which had $CaCO_3$ added at approximately the same rate as the Marion treatment, showed a final pH (7.4) the same as the Marion sludge treatment. The West Lafayette sludges had only a slight effect on soil pH because of a low inorganic C content and low application rate (25 mt/ha). Due to the length of the study (423 days), it is likely that inorganic C in the sludges had sufficient time to react thoroughly with the soil system.

It is possible to calculate the percent inorganic C lost by measuring the inorganic C content of the soil-sludge mixtures at the end of the study. As shown in Table 11.2, decreasing amounts of inorganic C were lost in the Anderson sludge treatments (89 to 24%) as the application rate increased from 40 to 200 mt/ha. Only 29% of the inorganic C in the Marion sludge was neutralized, a value similar to that for the control plus $CaCO_3$ (39%). For the Chicago, Frankfort and West Lafayette sludges, from 53 to 100% of added inorganic C was neutralized during the study. The final inorganic C levels of the soil-sludge mixtures were closely related to the final soil pH (Table 11.2).

Decomposition of Organic C

The decomposition of sludge was determined under field conditions by periodic measurement of CO_2 evolved from soils contained in PVC cylinders. Using such a system, sludge decomposition in soil will be partly controlled by daily and seasonal variations in soil temperature and moisture, variables which are carefully controlled in laboratory studies. The data collected under field conditions represents decomposition of sludges applied to soil during early September when soil temperatures and moisture content were favorable for rapid microbial growth. Obviously, the decomposition occurring during the initial three months after sludge addition will vary if soils are amended in summer, as opposed to winter.

Initially, all sludge treated and control soils had relatively high rates of CO_2 evolution. Evolution of CO_2-C ranged from 39 to 224 kg C/ha/day for the control and West Lafayette liquid (25 mt/ha) treatments, respectively (Table 11.3). The rates of CO_2 evolution from sludge amended and nonamended soils gradually decreased with time after application. The initial high rate of CO_2 evolution was due to rapid decomposition of easily degradable sludge organic materials and dissolution of $CaCO_3$ present in some of the sludges (Table 11.1). The initial rate of CO_2 evolution for the control soil (39 kg C/ha/day) was slightly higher than values reported for other mineral soils (Wildung *et al*., 1975; Kowalenko *et al*., 1978). The rate of CO_2 evolution observed in the control soil may be a response to the drying and wetting cycle used during preparation of the soil cylinders (Alexander, 1977).

The rate of CO_2 evolution from soils was a function of time after sludge application, soil temperature and moisture content. Differences in CO_2 evolution for the various sludge treatments are easily observable from the rate data presented in Table 11.3. This is also apparent when cumulative CO_2-C evolved is calculated from the rates for each sampling day (Figures 11.1 to 11.3). Initially, soil moisture and temperature (Table

11.3) appeared to influence CO_2 evolution; however, the individual effects of moisture and temperature were difficult to separate. Temperature appeared to be more of a limiting factor than soil moisture since water potential values were always within the optimum range of -0.2 to -1 bars (Sommers et al., 1981). The rapid decrease in the rate of CO_2 evolution with time was not entirely due to the gradual lowering of soil temperature because the rate of sludge decomposition typically decreases as the supply of easily decomposable organic C is exhausted. When soil temperatures were <2°C, essentially no CO_2 was evolved from the control or sludge treated soils. The influence of soil temperature on CO_2 evolution is also shown by an increase in CO_2 evolution when soil temperatures began to rise in the spring months (Figures 11.1 to 11.3; Table 11.3). The degradation of sludge organic C in the spring may also be enhanced by physical breakdown of sludge particles by freezing and thawing (Alexander, 1977), which occurred from December to March. With favorable soil moisture and temperature, CO_2 evolution for all treatments was nearly at the control level (Table 11.3) one year after sludge application demonstrating the presence of a resistant fraction in sludge organic C as noted in other studies (Gilmour and Gilmour, 1980; Reddy et al., 1980).

As discussed in the Materials and Methods section, total CO_2 evolved was calculated by assuming that the CO_2 evolved on a sampling date represented the average for the time period between measurements. The cumulative CO_2-C evolved over the 423 day study period ranged from 1764 kg/ha (control) to 5608 kg/ha (Anderson, 200 mt/ha). The sludges added at 100 mt/ha averaged 3273 kg/ha CO_2-C evolved while the West Lafayette wet and dry sludges averaged 4221 and 3705 kg/ha CO_2-C evolved, respectively (Table 11.4).

The proportion of sludge organic C decomposed varied with the source of sludge (properties shown in Table 11.1). Several methods were evaluated for calculating organic C decomposition based on either cumulative CO_2-C evolved or a direct measurement of organic C remaining in the soil after the study. Calculations based on the organic C remaining in the soil give the highest values for percent organic C decomposed (Table 11.4). The percentage of organic C decomposition ranged from 21 (Chicago 100 mt/ha) to 51% (Anderson 200 mt/ha; Frankfort 100 mt/ha) with the four sludges applied at 100 mt/ha averaging 36% decomposition (Table 11.4). In contrast to most previous studies, the decomposition of Anderson sludge increased from 28 to 51% as the application rate increased from 100 to 200 mt/ha. This result was not obtained with any of the other calculation methods for sludge organic C decomposition. These calculations based on a direct measurement of organic C remaining having limited meaning due to the fact that only three treatments have organic C levels significantly different ($p = .01$) from the control (Table 11.4).

The cumulative amount of CO_2-C evolved can be corrected for decomposition of organic matter in the untreated soil, ignoring CO_2 from dissolution of $CaCO_3$. After subtracting CO_2-C for the untreated control from each treatment, sludge organic C decomposition ranges from 13 (Chicago 100 mt/ha) to 41% (West Lafayette wet 25 mt/ha). The sludges applied at 100 mt/ha showed an average of 19% decomposition of added organic C. The percentage organic C decomposed for the Anderson sludge was the same at 40 and 100 mt/ha rates (25 and 24%, respectively) but decreased at the 200 mt/ha rate to 19% (Table 11.4).

Carbon dioxide resulting from decomposition of organic C can be corrected for inorganic C dissolution by using either the $CaCO_3$-control or a direct measure of inorganic C remaining in the soil at the end of the study. By proportioning CO_2 from the $CaCO_3$-control to the $CaCO_3$ added in each treatment, all three rates of the Anderson showed essentially the same degree of decomposition (17 to 22% of added organic C) as shown in Table 11.4. Decomposition of added organic C ranged from 8 (Marion 100 mt/ha) to 41% (West Lafayette wet 25 mt/ha) with the sludges applied at 100 mt/ha averaging 14%. Alternatively, by correcting cumulative CO_2-C evolved for inorganic C dissolution by direct measurement of inorganic C remaining after the experiment, the percent added organic C decomposition ranges from 0 (Marion 100 mt/ha) to 40% (West Lafayette wet 25 mt/ha). The Anderson, Frankfort and Chicago sludges applied at 100 mt/ha showed an average of 11% decomposition of added organic C (Table 11.4). It should be noted that correcting for inorganic C dissolution markedly affects the results of decomposition calculations for the highly calcareous Marion sludge (5.97% inorganic C).

In all methods of calculating percentage organic C decomposed, the results obtained for the Marion sludge should probably be ignored because the elevated $CaCO_3$ content causes any correction approach to result in significant errors. For the other treatments, as the $CaCO_3$ content of the sludges decrease, the accuracy of CO_2 evolution as an index of decomposition increases. Corrections of cumulative CO_2 evolution for inorganic C remaining in the soil are strongly influenced by analytical errors because the correction is based on the difference between two numbers. Similarly, the percentage of organic C decomposition calculated from soil organic C remaining is based on the difference between the treatment and control. It is probable that such estiamtes of organic C decomposition are the least reliable. The method used for measurement of organic C in soil has a precision of approximately 5%, a value within the increase in soil organic C caused by some sludge treatments. Whenever data is calculated from the difference between two numbers, the relative error is magnified if noncompensating errors occur in either the analysis or the experimental design. Any slight methodology error results in relatively large changes in percent decomposition calculations based on direct soil organic C analysis. Estimates of sludge decomposition based on direct soil analysis may not be as reliable as those based on direct measurement of CO_2 evolved even with the assumptions made in calculating cumulative CO_2 evolved. Since the temperature and moisture were relatively constant between CO_2 evolution measurements (Table 11.3), the overall CO_2 evolution rates should not have varied in each of the three decomposition phases.

In most studies concerning decomposition of sludge organic C under laboratory and field conditions, the priming effect has not been considered. The priming effect is the influence of added organic C on the decomposition of native soil organic matter. Terry et al. (1979) used a ^{14}C-labelled synthetic sludge and found that CO_2 evolution from native soil organic matter after sludge application was 1.63 times higher than the CO_2 evolution from the control soil. By extrapolating a regression line plotting cumulative CO_2 evolution versus rate of sludge application, Hsieh et al., (1981) calculated a priming ratio of 1.32 and 0.81 for activated and digested sludge, respectively, at 22°C. If a priming ratio greater than 1.00 is employed as a correction factor, the percentage of sludge organic C decomposed would decrease.

Prediction of Sludge Decomposition

Several workers have developed models for decomposition of sludge organic C by using laboratory incubation data to develop the appropriate rate constants. Data from field studies is not readily available to test the validity of such models. Using data from this field study, the model developed by Reddy et al., (1980) was evaluated. The model assumes first-order kinetics for each of three decomposition phases. For first-order kinetics,

$$C_2 = C_1 e^{-k_i t} \tag{1}$$

where C_1 = initial organic C
C_2 = final organic C
k_i = rate constant for phase i
t = time elapsed, days

Sludge decomposition was divided into three phases - rapid, intermediate and slowly decomposable phases, with first-order rate constants of k_1, k_2, k_3, respectively. Based on data obtained in a 112 day incubation study, a rate constant ($k_3 = 0.003\ \text{day}^{-1}$) was selected for the resistant phase of sludge organic C decomposition and was multiplied by constants to obtain the rate constants for the other decomposition phases. The three phases for the West Lafayette and Anderson sludges were 90 to 100%, 85 to 90% and < 85% of the organic C remaining for the rapid, intermediate and resistant phases, respectively. The rate constant of the resistant phase ($0.003\ \text{day}^{-1}$) was multiplied by 20 and 5 for the rapid and intermediate phases, respectively, for the Anderson sludge. The rate constant for the resistant phase was multiplied by 30 and 5 for the rapid and intermediate phases, respectively, of the West Lafayette sludges. The Frankfort, Marion and Chicago sludges decomposition phases were 96 to 100%, 93 to 96% and < 93% of the organic C remaining for the rapid, intermediate and resistant phases, respectively. The resistant phase k was multiplied by 20 and 5 to obtain rate constants for the rapid and intermediate phases, respectively.

The rate constant was also corrected for soil temperature and moisture. Reddy et al., (1980), summarized numerous decomposition studies and developed the following temperature and moisture correction relationships. For temperature:

$$k_i' = k_i \theta^{(T_2 - T_1)} \tag{2}$$

where k_i = rate at reference soil temperature (23°C)
k_i' = rate at soil temperature
T_1 = reference soil temperature
T_2 = actual soil temperature
θ = constant (1.07)

The value of $\theta(1.07)$ used by Reddy et al., (1980) was estimated from C decomposition rates reported by several workers.

The correction for soil moisture was based on water potential as follows:

$$F_m = 0.874 - 0.115 \ln w \tag{3}$$

where F_m = relative rate of decomposition at a particular moisture tension
w = moisture tension in bars

The value of k used in equation 1 is obtained by correcting the resistant fraction rate constant for temperature and moisture from:

$$k_i = k_i' \times F_m \tag{4}$$

where k_i = phase rate constant
k_i' = rate constant corrected for temperature
F_m = relative decomposition rate at given moisture tension

The rate constant is then multiplied by the appropriate factor depending on the decomposition phase.

Therefore, by correcting daily rate constants for temperature and moisture and phase of decomposition, it is possible to plot simulated versus measured values of CO_2-C evolution rates (Figures 11.4 to 11.6). The $CaCO_3$-control was used to correct the rate of CO_2 evolution for $CaCO_3$ dissolution. It can be seen that predicted rates of CO_2 evolution agreed well with measured values for all sludges, except the wet and dry forms of the West Lafayette sludge, from the time of application (September, 1978) until the following spring (May, 1979). The model significantly underestiamted CO_2 release for the West Lafayette sludge. This sludge, taken directly from an anaerobic digestor, appears to possess a much higher fraction of easily decomposable C than the other sludges obtained from drying beds.

The model did not predict the increase in CO_2 evolution noted in all sludges as soil temperatures increased in the early spring (Figures 11.4 to 11.6). During this period, all sludge treatments had CO_2 evolution rates greater than the control while the calculated rates were only slightly altered by temperature. The increased CO_2 evolution in the early spring was likely due to freezing and thawing altering the availability of both sludge and soil organic C to microbes (Alexander, 1977).

SUMMARY

The results indicate that CO_2 evolution data yields lower estimates of decomposition of sludges than direct measurement of soil organic C. Using CO_2 evolution data corrected for the control, anaerobically digested sludges applied at 100 mt/ha averaged 19% decomposition over the 423 day field study. Dewatering and drying of a liquid sludge (West Lafayette) decreased the extent of decomposition of an anaerobically digested sludge.

Data for the Anderson, Marion, Frankfort and Chicago sludges indicate that the degree and relative order of decomposition are similar in both laboratory and field studies. In any decomposition study, inorganic C present in the sludge causes considerable difficulty in differentiating between CO_2 evolved from organic and inorganic C, because of dissolution of inorganic carbonates present.

A model developed by Reddy _et al._, (1980), which uses first-order rate constants for each of three decomposition phases, was applicable for predicting sludge deomposition under field conditions except for the West Lafayette sludge. The model also fails to predict increased CO_2 evolution for control and sludge treated soils in the early spring as soil temperatures rise. This rapid increase in CO_2 activity in the early spring is likely due to the physical effects of freezing and thawing on increasing the availability of soil and sludge particles to soil microorganisms (Alexander, 1977).

Table 11.1. Properties of municipal sludges used in field study.

Sludge	Volatile solids	Inorg. C	Org. C	Inorg. N	Org. N
	%*				
Anderson	34.2	2.03	15.42	0.062	0.728
Marion	38.8	5.97	7.47	0.011	0.501
Frankfort	31.2	1.87	10.39	0.084	1.048
Chicago (Imhoff)	39.1	1.08	16.80	0.135	1.654
W. Lafayette (dry)	56.8	0.40	31.99	0.077	3.291
W. Lafayette (wet)	56.8	0.40	36.89	1.069	4.542

* air-dry weight basis.

Table 11.2. Application rate, final pH, final inorg. C content and % inorg. C lost for sludges applied to Chalmers soil in the field.

Sludge added	Application rate	Final pH	Final inorg. C content of soil + sludge	Inorg. C lost
	mt/ha		mg/kg	% of added
Control*	-	5.3	19	-
Control + $CaCO_3$	-	7.4	1670	39
Anderson	40	6.2	57	89
	100	6.9	460	48
	200	7.0	1240	24
Marion	100	7.4	1770	29
Frankfort	100	6.7	290	65
Chicago	100	6.4	230	53
W. Lafayette (dry)	25	5.0	34	65
W. Lafayette (wet)	25	5.2	13	100

* Initial soil control pH was 5.2.

Table 11.3. Rate of CO_2-C evolution from soil cylinders maintained under field conditions and amended with municipal sewage sludges (study initiated August 23, 1978).

Days after application	Sludge added and rate (metric tons/ha)								Control + $CaCO_3$*	Control+	Soil temp‡	Soil moisture§
	Anderson			Marion	Frankfort	Chicago	W. Lafayette	W. Lafayette				
	40	100	200	100	100	100	25 dry	25 wet				
	kg CO_2-C/ha/day										C	Bars
2	91	117	157	132	111	108	159	194	105	39	26.7	-0.87
6	62	105	173	67	70	82	153	224	46	26	28.9	-0.87
10	39	67	115	36	36	49	88	84	26	17	23.6	-0.89
13	55	113	158	39	44	62	125	87	25	22	24.8	-0.62
16	28	45	74	19	24	31	35	37	13	14	28.3	-0.78
21	26	45	70	22	21	26	27	25	17	13	18.9	-0.74
29	14	27	41	10	11	17	16	22	8	7	15.8	-0.89
37	10	16	26	7	10	12	12	17	5	6	14.5	-0.62
47	10	14	22	8	8	9	8	8	8	5	10.3	-0.76
65	8	11	18	6	5	7	4	5	5	4	8.4	-0.81
101	0	0	0	0	0	0	0	0	0	0	-0.9	-0.03
195	1	2	3	1	2	2	2	4	0	1	8.6	-0.29
226	8	12	12	10	8	10	10	12	6	4	13.1	-0.32
245	24	33	22	22	22	25	26	34	19	14	19.0	-0.46
292	10	15	21	11	11	11	11	12	10	8	21.9	-0.91
315	13	19	20	14	16	16	16	16	14	10	21.6	-0.58
356	5	7	8	7	7	6	9	10	7	5	20.6	-0.44
376	7	10	12	9	10	9	9	10	8	8	19.2	-0.97
404	3	4	5	3	4	4	4	3	3	3	13.6	-0.53
423	2	1	2	2	2	2	2	2	1	1	7.2	-0.91

* Soil received 4.97 g of $CaCO_3$-C.
+ Soil received no amendments
‡ Daily mean temperature at 10 cm.
§ Soil moisture content of upper 15 cm of soil.

Table 11.4. Application rate, final organic C content, CO_2-C evolved and organic C decomposition for sludges added to Chalmers soil in the field.*

Sludge added	Application rate	Final organic C content of soil + sludge†	Total CO_2-C evolved	Sludge org. C decomposed based on‡			
				Soil org. C	CO_2-C (Control)	CO_2-C (Control + $CaCO_3$)	CO_2-C (soil inorg. C)
	mt/ha	%	-kg/ha-	---------- % of org. C added ----------			
Control	-	2.61a	1764	-	-	-	-
Control + $CaCO_3$	-	2.62a	2391	-	-	-	-
Anderson	40	2.77a	2760	39	25	22	13
	100	3.07b	4156	28	24	21	18
	200	3.21b	5608	51	19	17	16
Marion	100	2.78a	2884	45	23	8	0
Frankfort	100	2.82a	2918	51	17	14	5
Chicago	100	3.16b	3132	21	13	11	9
W. Lafayette (dry)	25	2.78a	3705	49	37	37	36
W. Lafayette (wet)	25	2.81a	4221	48	41	41	40

* All data represent the average of 3 replications.

† Values followed by the same letter are not significantly different ($p = 0.05$).

‡ See Materials and Methods for discussion on calculations.

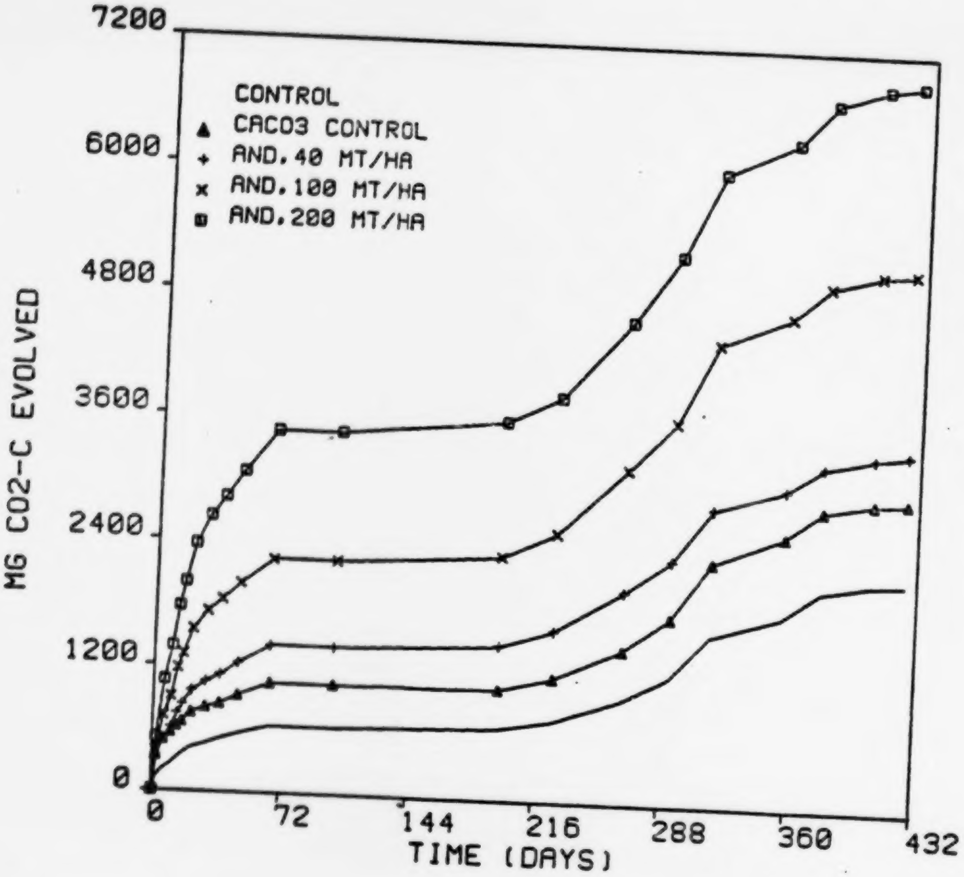

Figure 11.1. Cumulative CO_2-C evolution per column for the Chalmers soil control, Chalmers soil control plus $CaCO_3$ and for Chalmers soil amended at three rates with anaerobically digested sludge from Anderson, In.

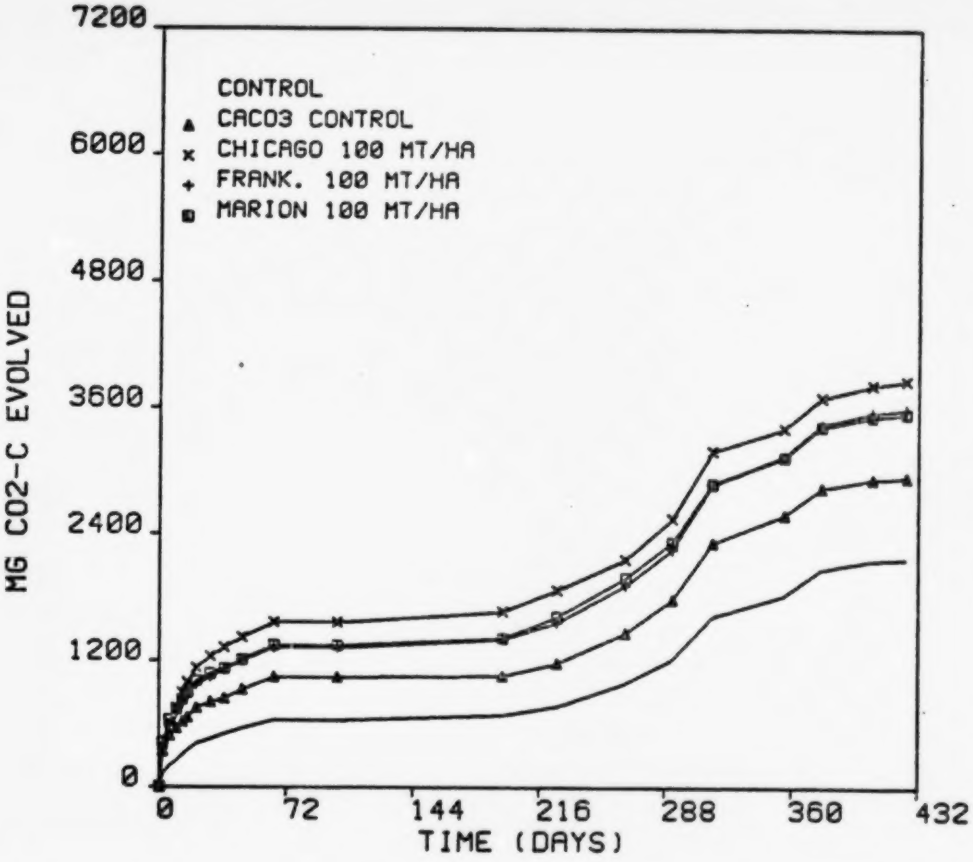

Figure 11.2. Cumulative CO_2-C evolution per column for the Chalmers soil control, Chalmers soil control plus $CaCO_3$ and for the Chalmers soil amended with anaerobically digested sludges from Chicago, IL, Frankfort and Marion, IN.

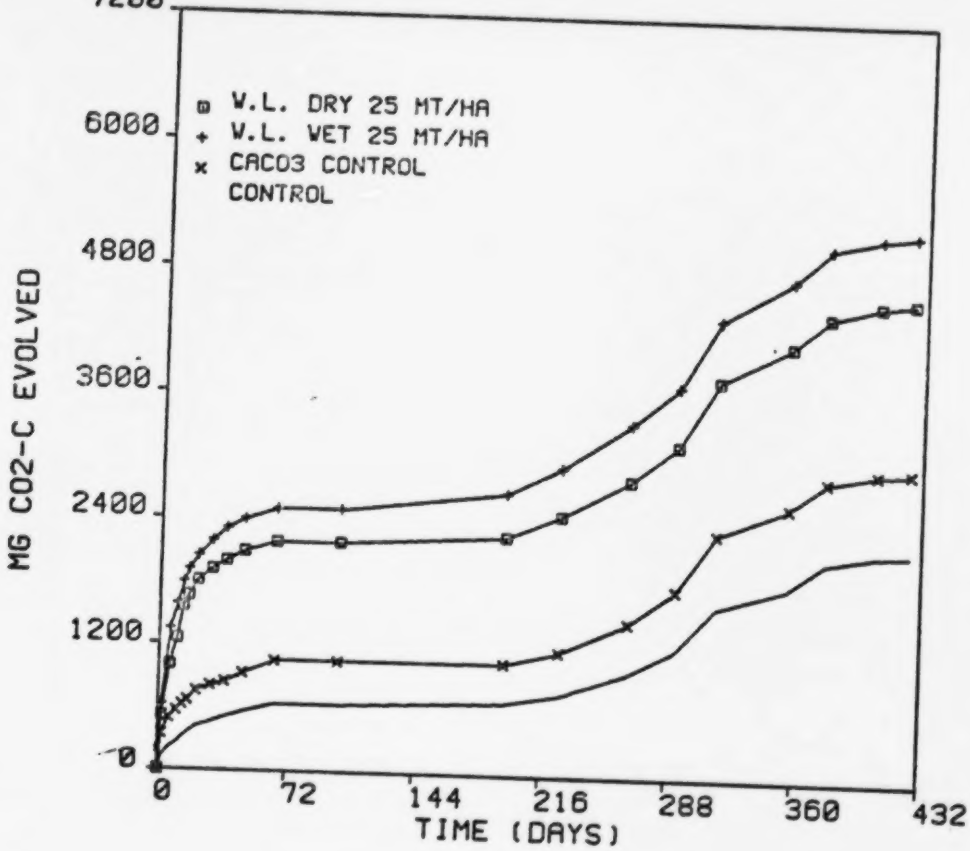

Figure 11.3. Cumulative CO_2-C evolution per column for the Chalmers soil control, Chalmers soil control plus $CaCO_3$ and for Chalmers soil amended with sludges taken directly from the anaerobic digestor at West Lafayette, IN, and applied wet or after dewatering and drying.

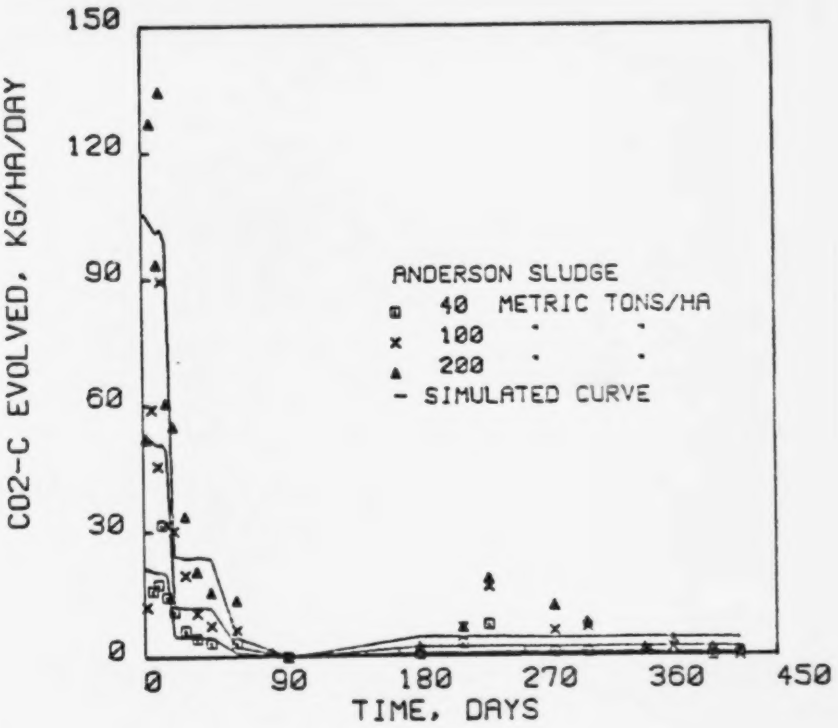

Figure 11.4. Rate of CO_2-C evolved for Chalmers soil amended at three application rates with anaerobically digested sludge from Anderson, IN. Solid line represents values predicted by first-order model.

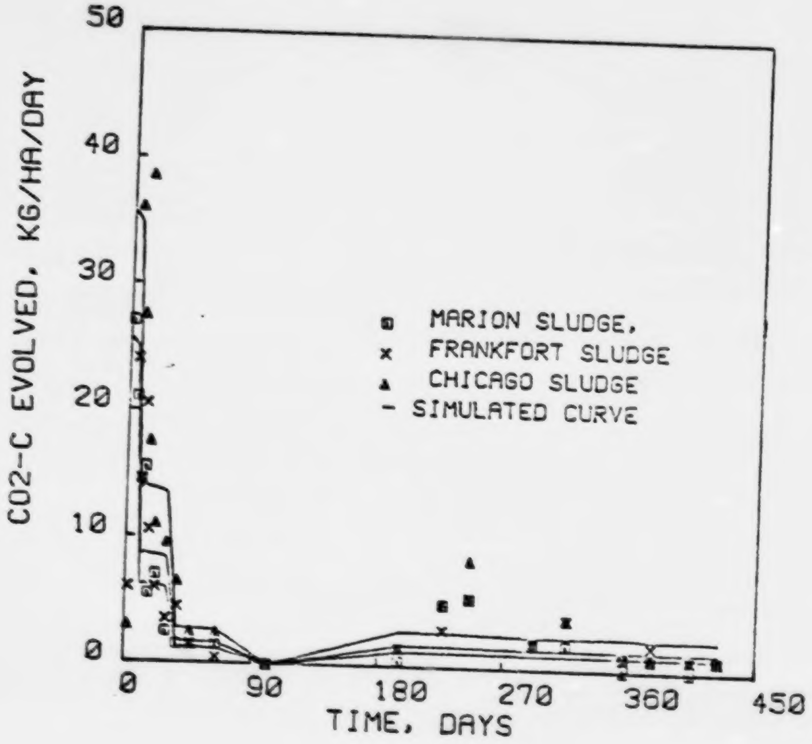

Figure 11.5. Rate of CO_2-C evolved for Chalmers soil amended at 100 mt/ha with anaerobically digested sludges from Frankfort and Marion, IN and Chicago, IL. Solid line represents values predicted by first-order model.

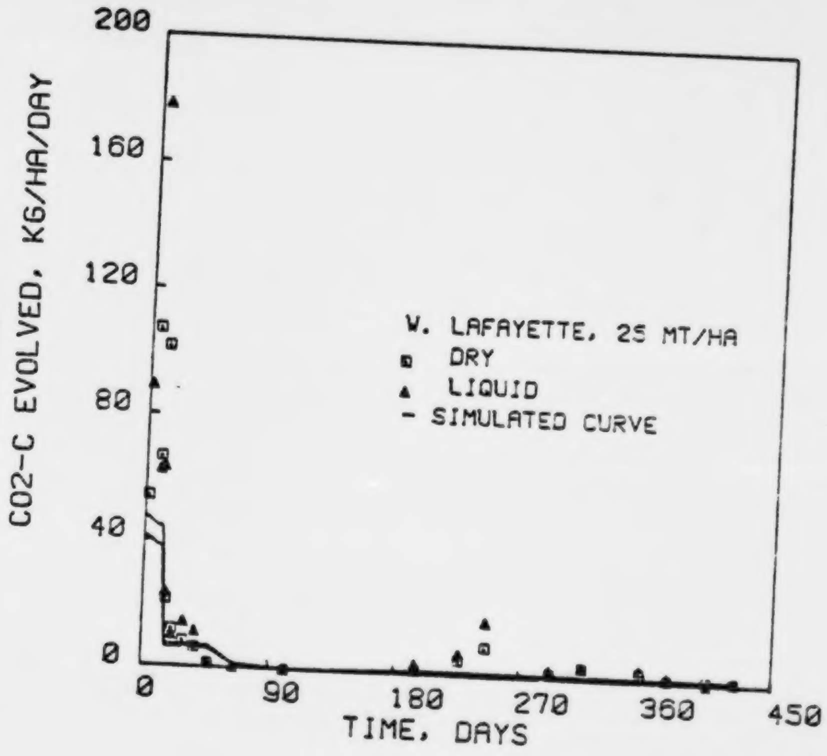

Figure 11.6. Rate of CO_2-C evolved for Chalmers soil amended with West Lafayette, IN, sludge taken directly from the anaerobic digestor and applied wet or dewatered and dried. Solid line represents values predicted by first-order model.

SECTION 12

THE DECOMPOSITION OF PHTHALATE ESTERS IN SOIL

INTRODUCTION

The application of sewage sludge on cropland not only provides crops with essential macro- and micro-nutrients but also may enrich soils with a variety of organic compounds that may be potentially toxic to either plants or animals. The only organic compounds considered by current federal regulations for sewage sludge disposal on cropland are the polychlorinated biphenyls (U.S. EPA, 1979). Another group of organic compounds that has been identified in sewage sludges are the phthalate esters. Characterization of the organic compounds in digested municipal sludges from three Indiana cities has revealed that phthalate esters (largely dibutyl phthalate) were present at concentrations of 10 to 1,000 mg/kg (Strachan *et al.*, 1982). Since dibutyl phthalate is used primarily as a plasticizer, industrial activities are likely sources for phthalates in sewage sludges. Natural sources of phthalates include tobacco leaves, grapes, and oxidized corn oil (Graham, 1973). Regardless of the source, the majority of phthalates entering sewage treatment facilities should be degraded during anaerobic digestion of sludge (Saeger and Tucker, 1976; Keyser *et al.*, 1976; Graham, 1973). However, as indicated, substantial amounts of phthalates have been found in some digested sludges.

Most reports on decomposition of phthalate esters in soils have been concerned with the loss of the plasticizer from buried plastic pipe (Booth and Robb, 1968; Decoste, 1968; Wendt *et al.*, 1970). While these studies report losses of phthalates from the plastic, the rates are generally slow. Soil respiration measurements have been used to study decomposition of dioctyl phthalate in a pastureland soil incubated at 48C (Eggins et al., 1971); however, it was not clear whether the entire compound was being metabolized or if only the octyl side-chain was being hydrolyzed and metabolized. In addition, studies have shown that phthalates can be taken up and accumulated by some plants and animals in model aquatic ecosystems (Metcalf et al., 1973). Because data are needed concerning the decomposition rates of these compounds in soil, the objective of this study was to identify the environmental factors that affect the decomposition of dibutyl phthalate in soil.

MATERIALS AND METHODS

Carboxyl-labeled ^{14}C monobutyl phthalate (MBP), and dibutyl phthalate (DBP) were synthesized from carboxyl-labeled ^{14}C phthalic acid (PA) (ICN

Chemical and Radioisotope Division). The specific activity of the ^{14}C labeled PA, MBP, and DBP was measured using a modified Allison (1960) technique whereby $^{14}CO_2$ liberated by H_2SO_4 - H_3PO_4 - $K_2Cr_2O_7$ digestion was trapped in 2.5-ml of NCS reagent (quarternary ammonium base in toluene supplied by Amersham/Searle Corp.) in a scintillation vial fitted with an absorption tower. The tower contents were washed into the vial with PPO-toluene cocktail (0.5% w/w 2,5 diphenyloxazole in toluene) and the ^{14}C activity was determined with a Packard 240 scintillation counter. Activity measurements were corrected for counter efficiency by assaying ^{14}C-toluene standards in a NCS matrix and dividing sample counts by the fractional proportion of activity measured in the standards. Relative specific activities for the compounds were: PA, 3.04×10^6 dpm/g; MBP, 1.05×10^7 dpm/g; and DBP, 2.85×10^7 dpm/g.

The incubation media used were: Chalmers silt loam (Typic Argiaquoll), Plainfield sand (Typic Udipsamment), Fincastle silt loam (Aeric Ochraqualf), silica sand (washed with 6 M HCl and then deionized H_2O until Cl^- free), and a mixture of 3/4 silica sand and 1/4 (w/w) Everglades peaty muck (Typic Medihemist). Properties of the soils are listed in Table 12.1. The anaerobically digested sludge used in this study was obtained from a drying-bed at the sewage treatment plant at Anderson, Indiana and contained 12.8% organic C, 0.66% organic N, 2.17% inorganic C and 0.07% inorganic N.

The experimental approach involved monitoring the evolution of $^{14}CO_2$ from soils treated with labeled phthalates. Soil or sand (100-g) was placed in a one liter Erlenmeyer flask and amended with the appropriate radioactive phthalate compound dissolved in acetone. The acetone was allowed to evaporate from the soil surface prior to initiation of the incubation. Table 12.2 lists the treatments (conducted in duplicate) used in the study. Most soils received 1-mg/g of phthalate compound. Soil moisture was adjusted to -0.6 bar tension with distilled water and 2 ml of a soil extract solution. The soil extract solution was prepared by shaking 50-g of Chalmers soil with 100-ml of water, centrifuging at 1,000 x g and collecting the supernatant. The soil extract was used to insure that adequate levels of soil microorganism were present in the incubation media at the start of the study. After the soil had been intimately mixed with the various amendments, the flasks were connected to a closed aeration system whereby CO_2-free, moist air flowed over the soil at a rate of approximately 8-ml/minute. Carbon dioxide evolved from the soil was trapped in 25-ml of 1 M KOH and analyzed for ^{14}C activity by acidifying a two-ml aliquot of the KOH trapping solution with 2 M HCl and collecting the $^{14}CO_2$ evolved in NCS reagent as described above.

RESULTS AND DISCUSSION

The decomposition of phthalate esters was determined from the amount of $^{14}CO_2$ evolved from soils amended with phthalates labeled in the carboxyl group. This approach should be valid since, according to the degradation scheme shown in Figure 12.1 (Englehardt et al., 1975), ^{14}C carboxyl-labeled PA, MBP, and DBP would release $^{14}CO_2$ only when either the aromatic ring had been cleaved and metabolized to CO_2 or protocatechuate had been formed. Protocatechuate added to soils is rapidly metabolized via the tricarboxylic acid cycle to CO_2. In both cases, the labelled carboxyl group is liberated at a point in microbial metabolic pathways where $^{14}CO_2$ evolution should indicate that complete degradation of phthalate has occurred. However, a small amount of ^{14}C may be incorporated into newly synthesized microbial biomass and, thus retained in the soil or into stable soil organic matter by chemical and/or enzymatic reactions. The results obtained are expressed as a percentage of added ^{14}C evolved as $^{14}CO_2$. Data from the sterile soil treatment indicated that almost all DBP decomposition was due to microbial processes in the soil (Table 12.3). The small amounts of phthalate degradation observed in sterile soils late in the incubation could be due to air-borne contaminants entering the incubation system.

Phthalic acid was rapidly decomposed after addition to Chalmers soil, whereas MBP and DBP were metabolized only after a lag period of approximately 18 days (Figure 12.2). After 53 days of incubation 100% of the PA had been decomposed, whereas only 46 and 39% of the MBP and DBP, respectively, had been degraded. Since hydrolysis of the ester linkage(s) in MBP and DBP must precede ring cleavage, hydrolysis of the second ester linkage appears to be the rate limiting step in DBP decomposition. Englehardt et al. (1975) reported similar results for DBP degradation by microbial culture, noting that DBP was rapidly converted to MBP; however, hydrolysis of the second butyl ester was relatively slow.

Dibutyl phthalate decomposition in soil was evaluated as a function of soil pH. From $^{14}CO_2$ evolution data (Table 12.3), it is clear that soil pH has an effect on the initial rate of DBP decomposition. At an initial soil pH of 7.4 (final pH = 6.8), DBP was rapidly degraded while in the same soil at pH 6.0, DBP decomposition was retarded. Since PA rapidly decomposed in this soil at pH 6.0 it appears that soil pH affects hydrolysis of the butyl ester rather than metabolism of the PA formed. Although it is difficult to generalize about the sensitivity of esterase enzymes to pH, the lower soil pH (6.0) may be responsible for the partial inactivation of the esterase and, therefore, result in less DBP and MBP decomposition. After an initial delay, the soil at pH 5.3 decompsed DBP more rapidly than the soil at pH 6.0 (Table 12.3). Acid hydrolysis of the ester linkage may have occurred at this pH, however, the initial lag phase implies that only microbial processes were involved in DBP degradation. Regardless of the effect of soil pH on initial decomposition rates, after 80 days of incubation the amount of DBP decomposed was similar for all pH treatments.

Decomposition of DBP was also affected by soil type and soil temperature (Table 12.3). Decomposition of DBP was significantly greater in the Chalmers soil (85%) than in the silica sand medium (0.1%). This may have been due to the greater microbial numbers and organic substrate available. Also soil clays are involved in catalyzing hydrolysis reactions and in stimulating bacterial activity (Stotzky and Rem, 1966), and, thereby, may have increased DBP degradation in the Chalmers soil. The decomposition of DBP at soil temperatures of 4, 23, and 30C was 2, 32, and 78% of the added DBP, respectively, after 53 days of incubation (Table 12.3). These data indicate that DBP may persist after addition to cropland during periods of low soil temperatures, but the compound will degrade during subsequent warmer months.

Decomposition of DBP was not affected by amending soils with NH_4^+ and $CaCO_3$ (Fig. 12.3). Although decomposition of phthalates requires an N source, apparently the soil supplied sufficient available N to the microbial population for DBP decomposition. The added $CaCO_3$ did not increase soil pH since protons generated during nitrification of added NH_4^+ essentially maintained the soil pH at the same value (pH 6.1 while the soil with no NH_4^+ or $CaCO_3$ amendments was pH 6.2).

Submerged soils amended with DBP initially decomposed DBP more rapidly than unsaturated soils (Table 12.3). This finding is in contrast to that reported by Johnson and Lulves (1975). The reason for increased decomposition under saturated conditions is not clear, particularly since ring cleavage of PA is typically an aerobic process. However, a greater concentration of DBP (water insoluble) could occur at the water-air interface allowing aerobic decomposition to occur. In addition, any PA formed from ester hydrolysis of the MBP and DBP could diffuse to the upper, aerobic water zone. The more gradual decomposition may indicate diffusion of the phthalate compound into an aerobic zone. In aerobic soils, increasing the amount of DBP added from one to four mg/g soil had no effect on DBP degradation rate (Table 12.3).

Addition of an organic substrate (sludge) had essentially no effect on DBP decomposition (Fig. 12.3). Apparently, the breakdown of DBP is not affected by either co-metabolism during the breakdown of the sludge materials or the macro- and micro-nutrients supplied by this organic waste. Sludge addition tended to decrease PA decomposition initially, but 100% decomposition was observed at completion of the study. However, addition of muck to the silica sand greatly increased decomposition of DBP (Table 12.3). Increasing the organic matter content of the sand from zero to approximately 25% probably stimulated microbial activity and provided an adequate environment for decomposition of DBP.

SUMMARY

Substantial amounts of dibutyl phthalate may be applied to soil through application of sludge. The decomposition of dibutyl phthalate in soils is affected by soil type, soil pH, and soil temperature. Increasing soil temperature and organic matter increase the rate of DBP degradation. Soil pH can affect the rate of cleavage of the ester linkage in MBP and, thereby delay the degradation of the compound. However, DBP was completely metabolized within 100 days in soils of pH 5.7. The decomposition of DBP under temperate winter conditions may be slow, but it will rapidly increase during the warmer spring and summer periods. In general, dibutyl phthalate should not persist in the majority of soils for time periods > 100 days. It should be emphasized that the amounts of phthalates added to soils in this study were considerably greater than those encountered for systems applying sludges at rates consistent with the nutrient requirements of crops. The application of a sludge containing 1% DBP at 30 metric tons/ha (a typical sludge application rate?) would add 300 kg DBP/ha while the low rate of DBP used in this study corresponds to ~ 2000 kg DBP/ha. Since essentially complete degradation of DBP occurred within 100 days, application of sludges containing < 1000 mg phthalates per g to cropland will not constitute a long-term environmental concern, realizing that plant uptake data is needed to evaluate the overall impact of sludge-borne phthalates on crop production.

Table 12.1. Properties of soils used.

Soil	Sand	Silt	Clay	pH	Total C	Total N	CEC
	%						meq/100 g
Fincastle	13.5	69.7	16.8	5.9	1.24	0.108	15.2
Plainfield	90.3	7.1	2.6	6.2	1.38	0.121	9.3
Chalmers[+]	14.3	60.9	24.8	5.3	1.96	0.167	23.8
Everglades peaty muck	-	-	-	7.4	49.6	3.710	-
Silica sand	100	-	-	7.2	-	-	-

[+] Chalmers soil of pH 6.0 and 7.4 were also used.

Table 12.2. Soils, amendments, and conditions used in this study.

Soil	Amendment					
	Phthalate*	Amount	$(NH_4)_2SO_4$-N	$CaCO_3$	Sewage sludge	Other treatment+
		mg/g				
Chalmers	PA	1	0.54	3.65	-	-
Chalmers	PA	1	0.54	3.65	20	-
Chalmers	MBP	1	0.54	3.65	-	-
Chalmers	DBP	1	0.54	3.65	-	-
Chalmers	DBP	1	-	-	-	-
Chalmers	DBP	1	0.54	3.65	20	-
Chalmers	DBP	1	0.10	-	-	-
Chalmers	DBP	1	0.10	-	-	Sterile
Chalmers	DBP	1	0.10	-	-	Saturated
Chalmers	DBP	1	0.10	-	-	4°C
Chalmers	DBP	1	0.10	-	-	30°C
Chalmers	DBP	1	0.10	-	-	pH 5.3
Chalmers	DBP	1	0.10	-	-	pH 7.4
Chalmers	DBP	4	0.10	-	-	-
Sand	DBP	1	0.10	-	-	pH 7.2
Sand + muck	DBP	1	0.10	-	-	pH 7.4
Plainfield sand	DBP	1	0.10	-	-	pH 6.2
Fincastle	DBP	1	0.10	-	-	pH 5.9

* PA, phthalic acid; MBP, monobutyl phthalate; DBP, dibutyl phthalate

+ Unless specified, soil used was Chalmers pH 6.0 prior to treatment and amended samples were incubated at 23°C.

Table 12.3. Decomposition of dibutyl phthalate as affected by soil type, pH, temperature, aeration status, and sterilization.

Soil	Treatment	Incubation time, days							
		3	6	11	18	32	53	80	200
		Percent of added activity evolved as $^{14}CO_2$-C							
Silica sand	-	0.08	0.1	0.1	0.1	0.1	0.1	0.1	0.6
Muck + sand	-	0.13	1.2	20.1	58.8	86.5	96.3	99.4	102.8
Plainfield	-	0.14	0.1	0.2	0.3	0.6	2.6	77.9	89.6
Fincastle	-	0.12	0.2	0.3	0.7	1.7	8.7	89.4	93.0
Chalmers (pH 6.0)	Sterile	0.01	0.02	0.04	0.1	0.2	0.4	1.4	2.4
Chalmers (pH 6.0)	Anaerobic	0.04	0.6	6.6	12.3	45.1	68.6	83.7	97.8
Chalmers (pH 5.2)	-	0.09	0.2	0.8	1.7	39.3	95.4	97.0	98.6
Chalmers (pH 6.0)	-	0.13	0.3	0.8	1.6	4.8	32.4	84.7	88.1
Chalmers (pH 7.2)	-	1.20	16.5	63.3	78.0	90.8	94.9	95.8	97.2
Chalmers (pH 6.0)	30°C	0.13	0.4	1.4	2.7	9.8	77.7	82.6	‡
Chalmers (pH 6.0)	4°C	0.02	0.06	0.3	0.8	1.2	1.9	‡	‡
Chalmers (pH 6.0)	4X†	0.05	0.1	0.3	0.6	1.7	36.4	97.2	110

* All treatments received 1 mg DBP/g soil

† Chalmers soil treated with 4 mg DBP/g soil

‡ System lost due to mechanical failure

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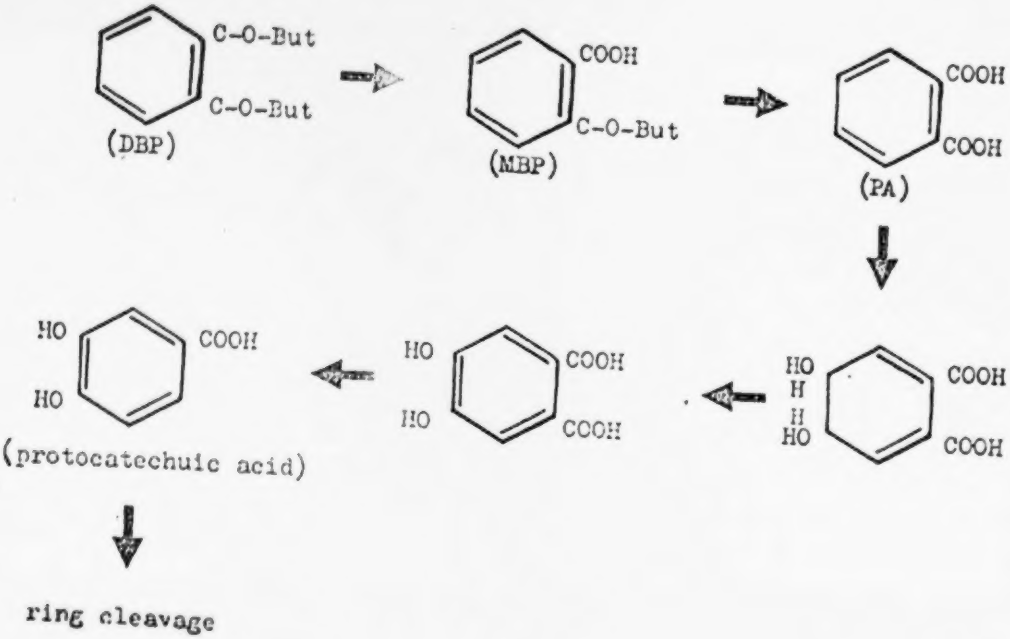
C-O-But
C-O-But
(DBP)
COOH
C-O-But
(MBP)
COOH
COOH
(PA)
HO
H
H
HO
COOH
COOH
HO
HO
COOH
COOH
HO
HO
COOH
(protocatechuic acid)
ring cleavage

Figure 12.1. Degradation pathway for DBP (Keyser et al., 1976). (But = $CH_3C_H-CH_2-C-$).

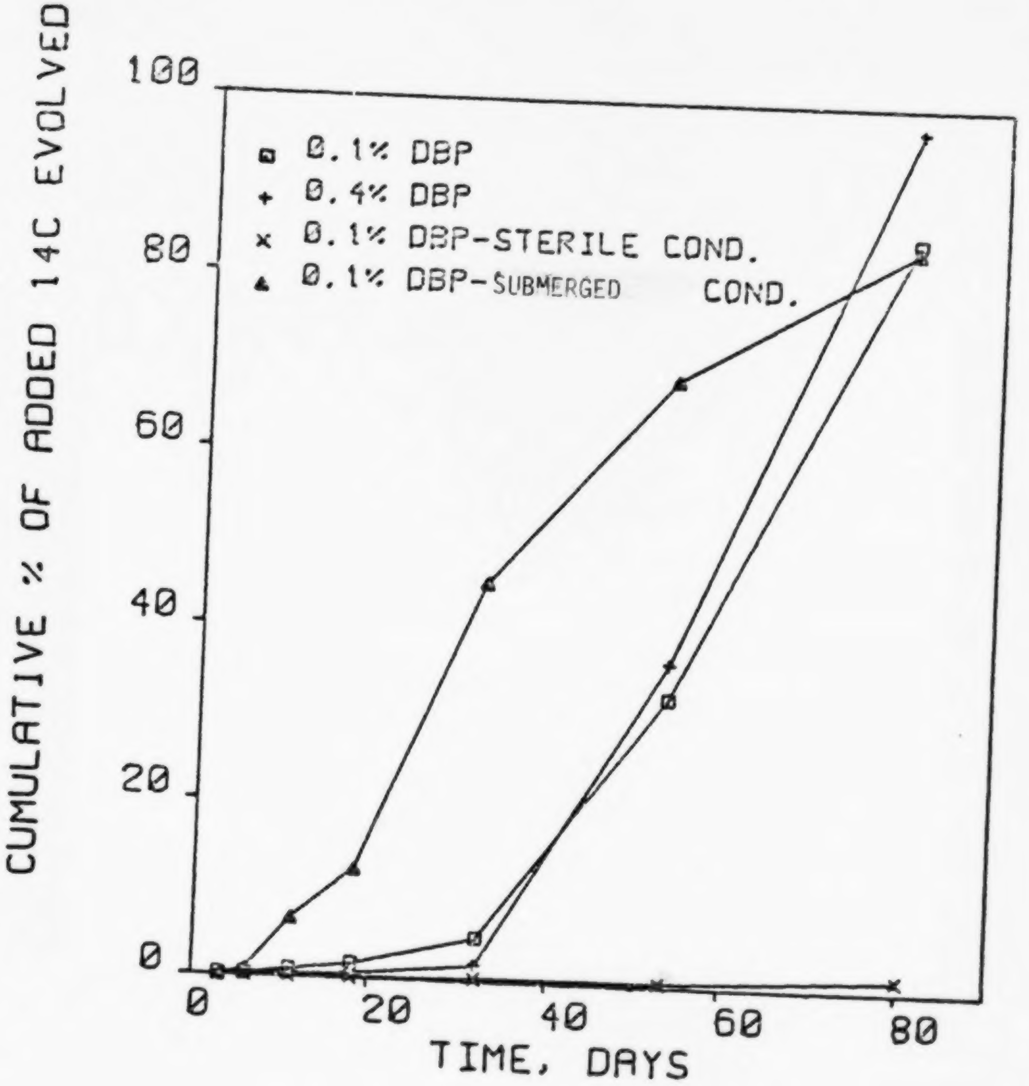

Figure 12.2. Effect of application rate, sterile and saturated conditions on decomposition of ^{14}C labelled DBP in a Chalmers soil incubated for 80 days.

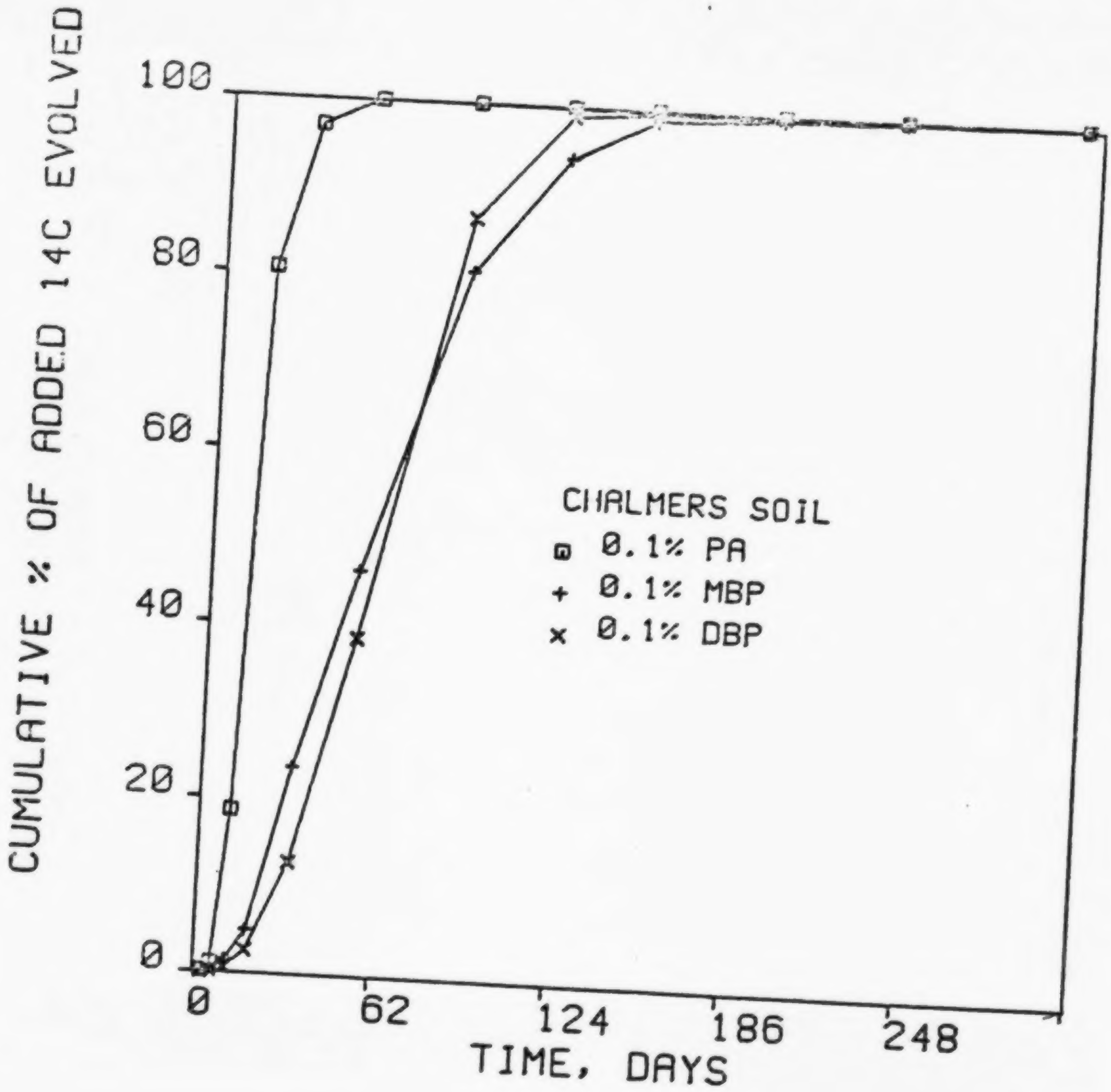

Figure 12.3. Decomposition ^{14}C labelled PA, MBP, and DBP in a Chalmers soil incubated for 305 days.

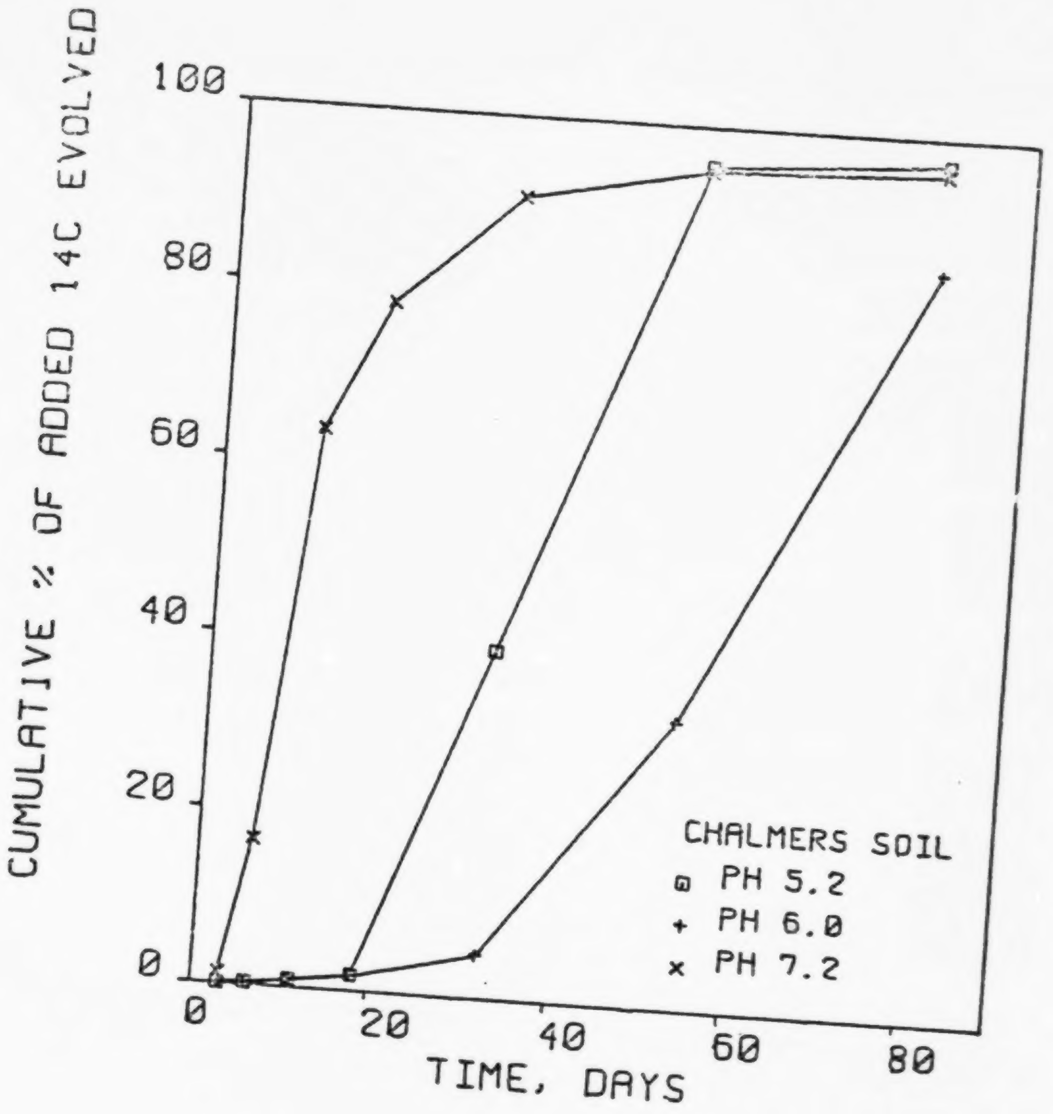

Figure 12.4. Effect of soil pH on decomposition of ^{14}C labelled DBP in Chalmers soils incubated for 80 days.

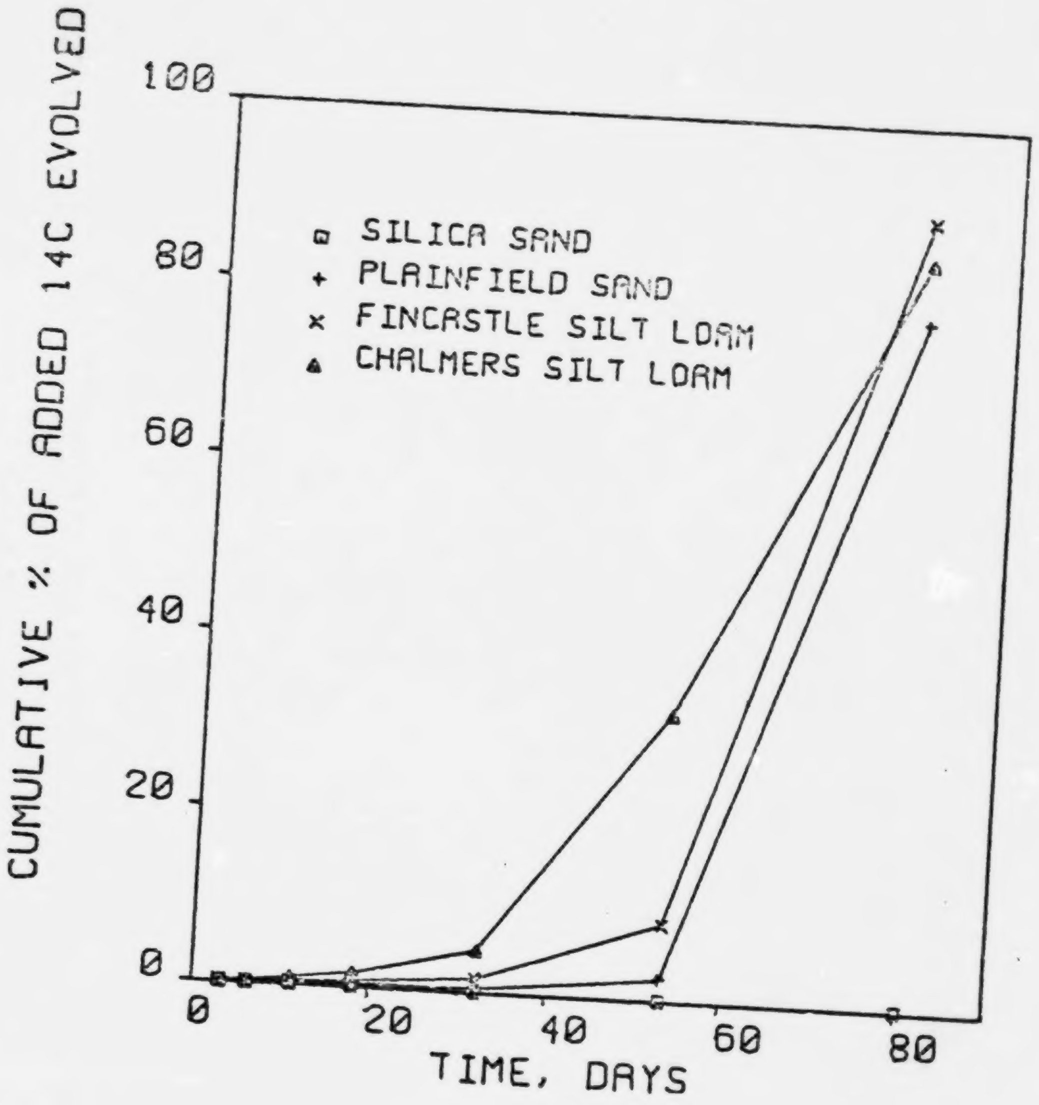

Figure 12.5. Effect of soil type on decomposition of ^{14}C labelled DBP.

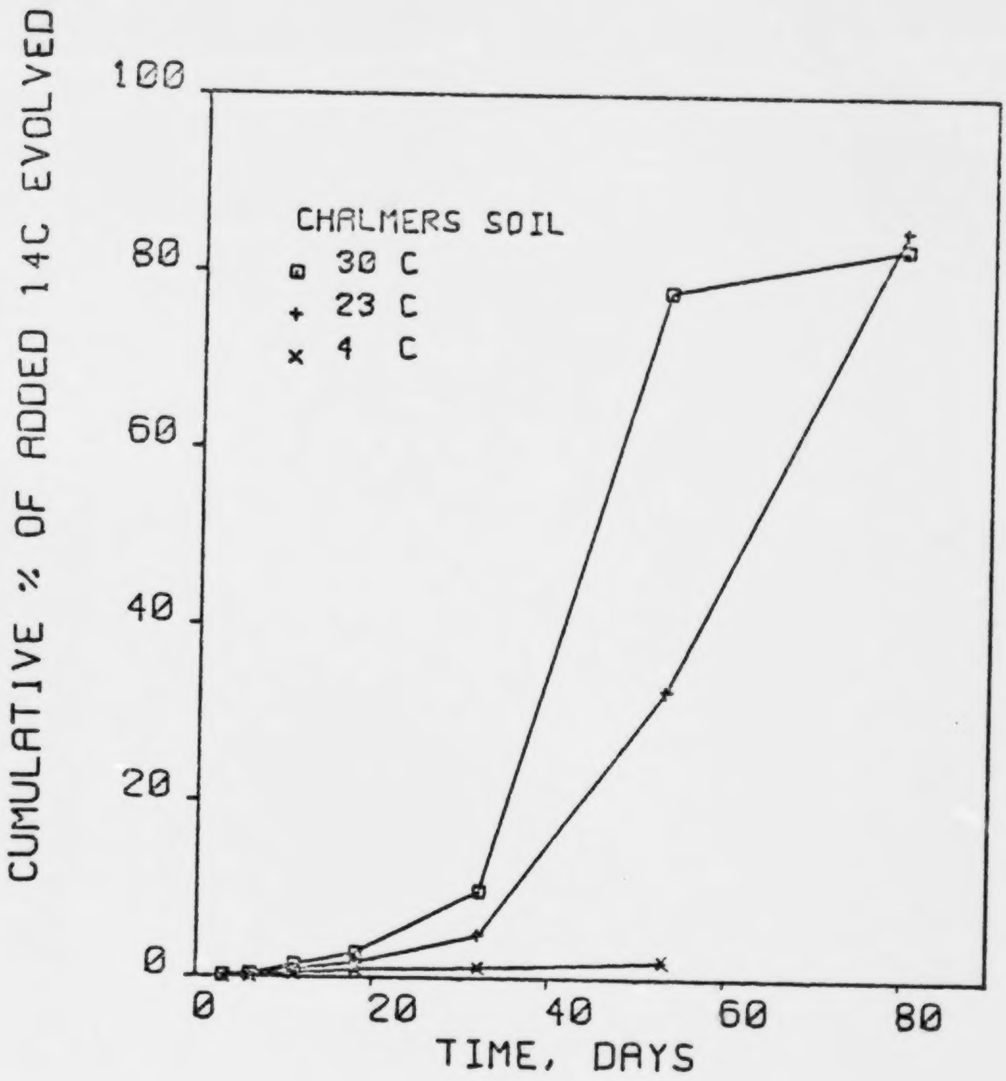

Figure 12.6. Effect of temperature on decomposition of ^{14}C labelled DBP in a Chalmers soil incubated for 80 days (4C sample was lost after 53 days).

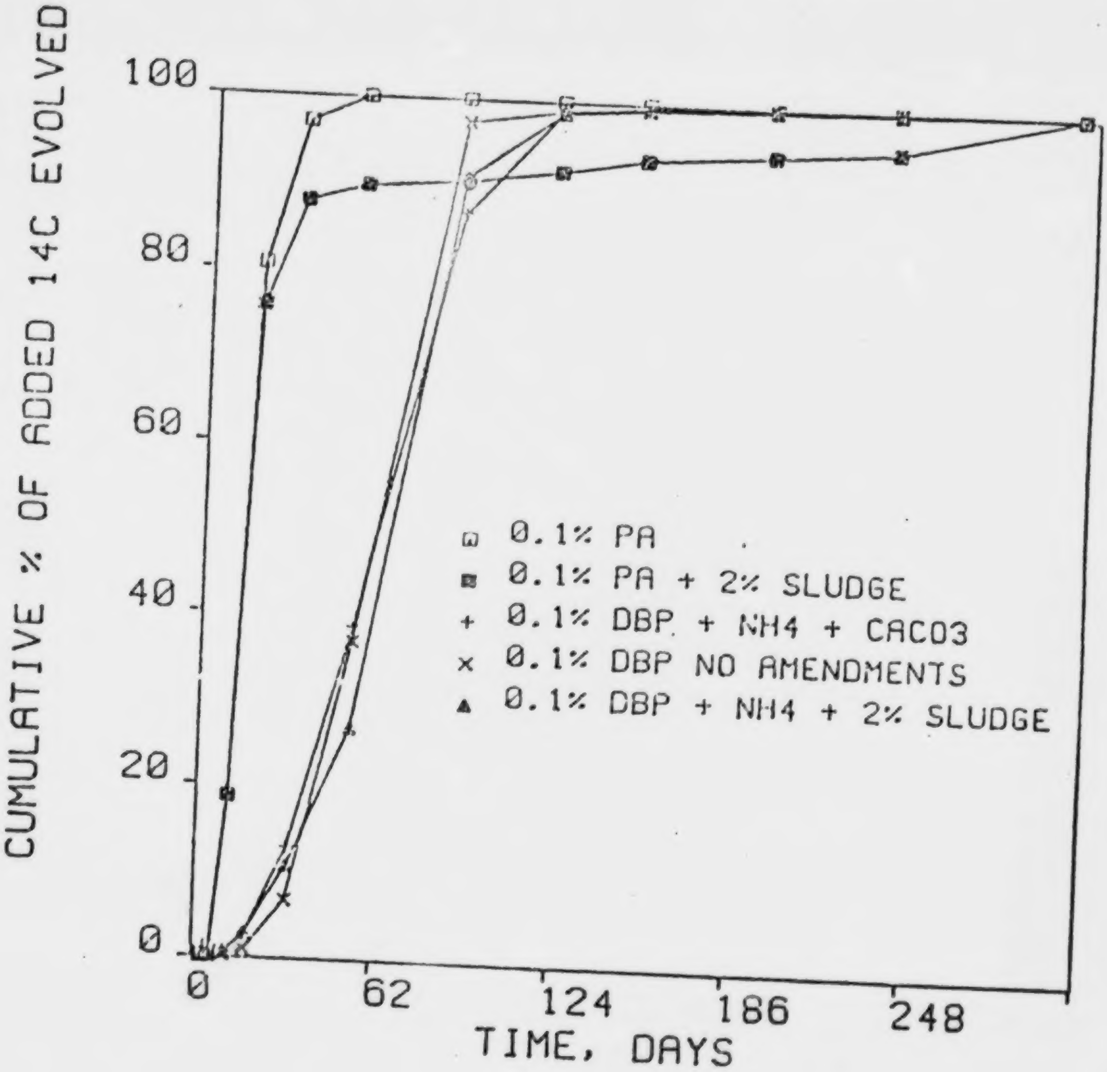

Figure 12.7. Effect of sludges and $(NH_4)_2SO_4$ + $CaCO_3$ amendments on PA and DBP decomposition in a Chalmers soil incubated for 305 days.

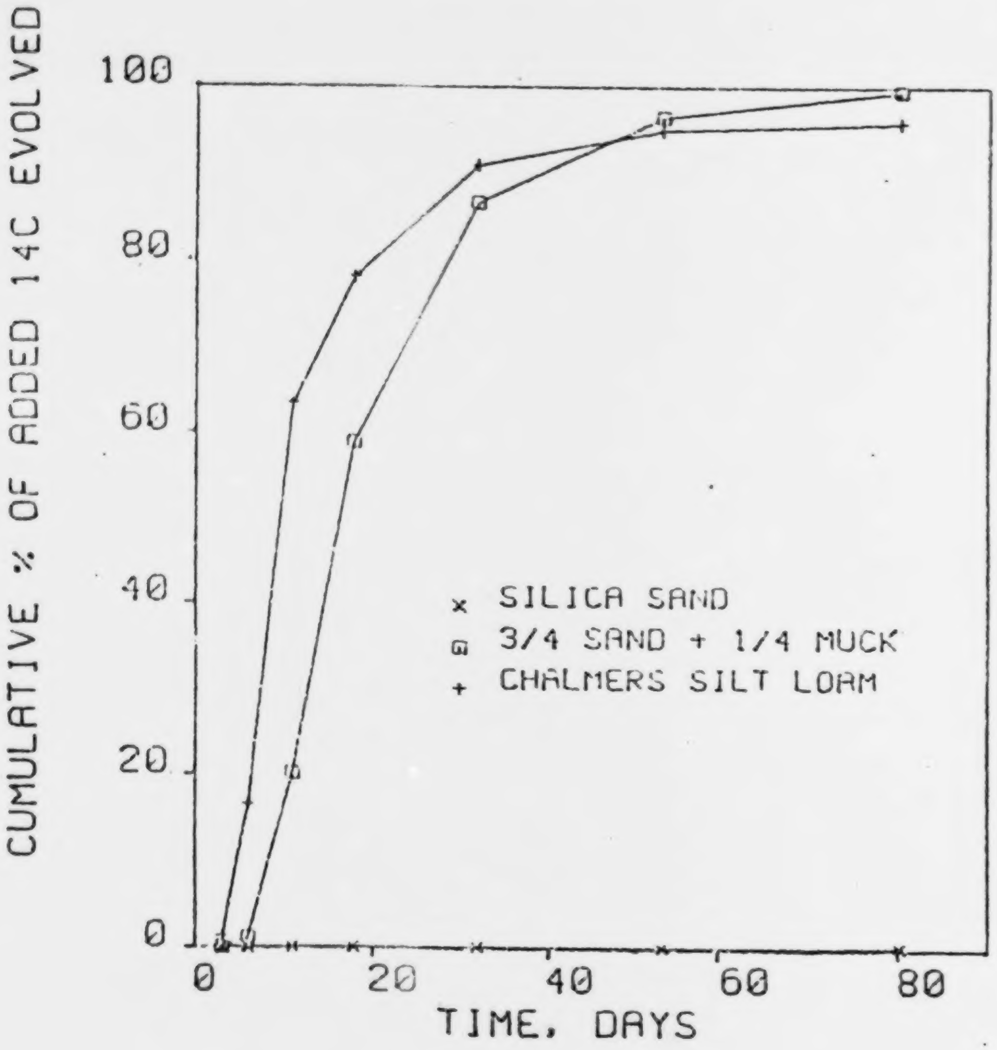

Figure 12.8. Decomposition of ^{14}C labelled DBP in Chalmers soil, silica sand and silica sand + muck soil.

SECTION 13

RETENTION OF HEAVY METALS BY SEWAGE SLUDGE

INTRODUCTION

Metals, such as Cd, Cu, Zn, Ni and Pb, are one of the major constraints associated with applying sewage sludges on agricultural cropland. The above metals can be present in sludges in a variety of chemical forms, including discrete crystalline or amorphous precipitates, complexes with organic matter, species adsorbed on the surface of oxide or carbonate minerals and association with the cation exchange complex. Because the forms of metals present can influence the potential for plant uptake following land application, knowledge on metal speciation in sludges is needed. The presence of adsorbed metals in sludges prompted the initiation of this study to evaluate the potential for and mechanisms of metal retention by sludges.

MATERIALS AND METHODS

The sludges used in this study were obtained from the Anderson, Frankfort, Marion and West Lafayette, Indiana, sewgae treatment plants (see Table 11.1).

General Procedures for Studies of Metal Retention by Sludges

The retention capacities of sludges, sludge fractions, and extracted sludges for metals were determined as follows unless otherwise specified. One gram (oven-dry weight) sludge samples were weighed into 50 ml Oak Ridge polyethylene centrifuge tubes, 20 ml of 0.01 M $Ca(NO_3)_2$ solutions containing from 0 to 2.5 mg/ml of Cd, Cu, Ni or Zn were added, and the mixtures were equilibrated for 18 hours on a reciprocating shaker. All metals were added as nitrate salts. After equilibration the tubes were centrifuged for 15 minutes at 12,000 x g, the supernatants were decanted into glass vials, and the pH of the supernatants determined by glass electrode (Peech, 1965). The sludge pellets were then sequentially extracted with 20 ml of 0.01 M $Ca(NO_3)_2$, 10 ml of DTPA, and 20 ml of 4 M HNO_3. The DTPA reagent employed was described by Lindsay and Norvell (1978), and contained the following: 0.005 M diethylenetriamine pentaacetic acid (DTPA), 0.01 M $CaCl_2$, 0.1 M triethanolamine (pH was adjusted to 7.3 with HCl). After addition of each extractant the mixtures were shaken for 18 hours, centrifuged for 15 minutes at 12,000 x g, and the supernatants were decanted. Cadmium, Cu, Ni, or Zn was then determined in the equilibrium solutions and $Ca(NO_3)_2$, DTPA, and HNO_3 extracts by atomic adsorption spectrophotometry. Metals retained by sludge were calculated as the difference in the amounts of metal added and that in the equilibrium solution.

Acidification of Frankfort Sludge

To determine the effects of $CaCO_3$ in sludge on metal retention, a comparison of the metal sorption capacities of acidified and natural sludge was conducted. Frankfort sludge was equilibrated with 30 ml of 1 M NaOAc (pH 5.0) according to the procedure outlined by Silviera *et al*. (1977) to remove free $CaCO_3$. The sludge was then adjusted to pH 4.5 by equilibration with an acetic acid-sodium acetate buffer. The metal retention properties of the acidified sludge were determined as previously described.

Density Fractionation of Sludges

Anderson and Frankfort municipal sludges were separated into heavy and light fractions by a heavy liquid technique as described previously (Section 4) to provide information on the sludge components involved in metal retention. The adsorptive capacity of the Anderson and Frankfort heavy and light fractions for Cd and Cu was determined by equilibrating subsamples of the heavy and light fractions with 0.01 M $Ca(NO_3)_2$ solutions, (4 ml/.2 g), containing varying concentrations of Cd or Cu. The concentrations of Cd and Cu in the $Ca(NO_3)_2$ solutions ranged from 0 to 2.0 mg/ml (0 to 40 mg/g sludge fraction). After equilibration, the supernatant was decanted and transferred to 5 ml glass vials, and the pH was measured by glass electrode. The supernatant was then transferred to 10 ml volumetric flasks and taken to volume with 0.01 M $Ca(NO_3)_2$. The heavy and light sludge fractions were then sequentially extracted with 4 ml of 0.01 M $Ca(NO_3)_2$, 3 ml DTPA, and 4 ml of 4 M HNO_3. After addition of each extract the sludge suspensions were shaken for 18 hours, centrifuged and the supernatant decanted into 10 ml volumetric flasks. The volumetrics were diluted to volume with 0.01 M $Ca(NO_3)_2$, DTPA or 4 M HNO_3. Metals in the original equilibrium solution and the three extracts were determined as described earlier.

Selective Removal of Sludge Components

The characterization of Cd, Cu, Ni, and Zn retention sites in sewage sludge was conducted by utilizing five independent extraction procedures: (1) 0.5 M NH_4F, (2) 0.3 M Na Citrate - 1 M $NaHCO_3$ - 0.12 M $Na_2S_2O_7$ (CDB), (3) 0.1 M $Na_4P_2O_7$, (4) 1 M sodium acetate (NaOAc) and (5) 30% H_2O_2. The preceding independent extraction techniques also evaluated the amount of Cd, Cu, Pb, Ni and Zn associated with each inorganic or organic sludge component.

Anderson sludge was subjected to a 0.5 M NH_4F extraction as adopted by Jackson (1970). One gram samples of Anderson sludge were extracted with 20 ml of 0.5 M NH_4F at pH 8.2. All extractions were conducted in 50 ml Oak Ridge screw-cap centrifuge tubes. After shaking for 24 hours on a reciprocating shaker, the tubes were centrifuged for 10 minutes at 12,000 x *g*. The supernatant was then decanted and transferred to glass vials and the pH determined. The supernatant was then analyzed for Cd, Cu, Pb, Ni, Zn, Al, Ca, Fe, and Mn by atomic adsorption spectrophotometry. The metal retention properties of the NH_4F-extracted Anderson sludge were determined as previously described.

Anderson, Frankfort and West Lafayette sludges were extracted with 0.3 M Na Citrate - 1 M $NaHCo_3$ and 0.12 M $Na_2S_2O_7$ (CDB) as developed by Jackson (1970). One gram of sludge was suspended in 32 ml of 0.3 M Na Citrate and 4 ml of 1 M $NaHCO_3$. The suspension was heated in a water bath at 80°C. To the heated suspension, 8 g of $Na_2S_2O_7$ were added with rapid stirring. The suspension was kept at 80°C for 15 minutes and then centrifuged at 10,000 x g for 15 minutes. All extractions were conducted in 50 ml Oak Ridge screw-cap centrifuge tubes. The supernatant was then decanted into a 50 ml volumetric flask, the pH measured and then taken to volume with Na citrate (0.3 M) - Na_2CO_3 (1 M). The CDB extract was then analyzed for Cd, Cu, Pb, Ni, Zn, Al, Ca, Fe, and Mn by atomic adsorption spectrophotometry. The metal retention properties of the CDB-extracted Anderson, Frankfort and West Lafayette sludges were determined as previously described.

Anderson and Frankfort sludges were extracted with 0.1 M $Na_4P_2O_7$ (pH 6.5) to solubilize humic materials (Stover et al., 1976). One gram subsamples of Anderson and Frankfort sludge were amended with 40 ml of 0.1 M $Na_4P_2O_7$. All extractions were conducted in Oak Ridge screw-cap centrifuge tubes. After shaking for 16 hours on a reciprocating shaker, the tubes were centrifuged for 10 minutes at 12,000 x g. The supernatant was then decanted and transferred to a 50 ml of volumetric flask, the pH determined, and the extract diluted to volume with 0.1 M $Na_4P_2O_7$. The extract was then analyzed for Cd, Cu, Pb, Ni, Zn, Al, Ca, Fe, and Mn by atomic adsorption spectrophotometry. The metal retention properties of the $Na_4P_2O_7$-extracted Anderson and Frankfort sludges were determined as previously described.

Anderson and Frankfort sludges were subjected to a 1 M NaOAc treatment to destroy carbonates according to the procedure outlined by Silviera, Sommers and Nelson (1977). One gram subsamples of air-dried Anderson and Frankfort sludges were treated with 25 ml of 1 M NaOAc (pH 5) and digested for 30 minutes on a hot plate with intermittent stirring. Following digestion, the suspension was transferred to 50 ml Oak Ridge type screw-cap centrifuge tubes and centrifuged for 10 minutes at 10,000 x g. The supernatant was decanted and transferred into a 100 ml volumetric flask. The residue was washed twice by resuspension in 1 M NaOAc and centrifugation. The pH of each solution was measured and the extract diluted to volume with 1 M NaOAc. Each solution was analyzed for Cd, Cu, Pb, Ni, Zn, Al, Ca, Fe and Mn by atomic adsorption spectrophotometry. The metal retention properties of the NaOAc-extracted Anderson and Frankfort sludges were determined as previously described.

Anderson and Frankfort sludges were subjected to a H_2O_2 treatment to oxidize organic matter as outlined by Jackson (1970). To one-gram samples of Anderson and Frankfort sludges, 10 ml of H_2O and 4 ml of 0.1 N HCl were added. An additional 4 ml of 0.1 N HCl were added for each 1 percent of $CaCO_3$ present. The suspension was stirred to facilitate reaction. The 250 ml tall form beakers containing the H_2O - 0.1 N HCl-sludge suspension were then placed on a hot plate and warmed for one hour. Litmus paper was used to indicate pH. For pH's greater than 5.8, additional 4 ml increments of 0.1 N HCl were added until the pH was below pH 5.8. To the 250 ml tall-form beakers containing the slightly acid sludge suspension, 10 ml of 30% H_2O_2 were added, the beakers were covered with a tightly fitting watch glass and the samples digested on a hot plate. To avoid frothing, the hot plate had to be regulated continuously. From time to time the suspensions were mixed by rotation of the beaker. The H_2O_2 digestion was continued for four days. Additional H_2O_2 was added after the initial H_2O_2 had decomposed. The four day digestion period was necessary to ensure complete oxidation of the organic matter.

After complete oxidation of the organic matter, the cover glass and beaker were scrubbed with a rubber policeman and the contents of each beaker rinsed with H_2O_2 into a 50 ml Oak Ridge type screw-cap centrifuge tube. The tubes were centrifuged for 15 minutes at 12,000 x g. The supernatant was then decanted and transferred to 25 ml volumetric flasks, and the pH was determined by glass electrode. The extract was then diluted to volume with H_2O_2. Each extract was analyzed for Cd, Cu, Pb, Ni, Zn, Al, Ca, Fe and Mn by atomic adsorption spectrophotometry. The metal reaction properties of the H_2O_2 extracted Anderson and Frankfort sludges were determined as described earlier.

Solubility of Cd, Cu, Ni and Zn as Affected by pH

Cadmium, Cu, Ni and Zn solubility at different pH values was evaluated by equilibrating 0.05 N $Ca(OH)_2$ with 0.01 M $Ca(NO_3)_2$ solutions containing metal concentrations varying from 0 to 1 mg/ml. All equilibrations were conducted in 50 ml Oak Ridge screw-cap centrifuge tubes. The precipitation of metals was quantified by equilibrating 0 to 5 ml of 0.05 N $Ca(OH)_2$ with 5 ml of metal solutions containing 0.2, 0.25, 0.5 or 1.0 mg of metal per ml. After shaking for 18 hours on a reciporcating shaker, the tubes were centrifuged for 15 minutes at 12,000 x g. The supernatant was then decanted and transferred to glass vials. The solution pH was measured by glass electrode. Metal concentrations in the equilibrium solution were determined by atomic adsorption spectrophotometry.

RESULTS AND DISCUSSION

Retention of Metals by Untreated Calcareous Sludges

Sludges have a large capacity to sorb and retain added metals even though significant amounts of native metals are present in sludge. The retention capacity and the sites of metal retention may be significant in determining the release of metals to the soil. The retention capacity and sites of metal retention could provide information which would enable more accurate predictions of metal behavior in soils. The following discussion is limited to results obtained for Cd; data are also presented for Zn, Cu and Ni.

Table 13.1 presents the retention characteristics of added Cd by air-dried Frankfort sludge. Approximately 86 to 97% of the added Cd was retained by Frankfort sludge at the lower metal concentrations (2 to 20 mg/g). At the higher Cd addition rates (30 to 50 mg/g), 53 to 75% of the added Cd was retained. Table 13.2 shows the retention of Cd by air-dried

Anderson sludge. At the low Cd addition rates (2 to 20 mg/g), 72 to 95% of the added Cd was retained, whereas approximately 42 to 58% of the added Cd was retained at the higher addition rates (30 to 50 mg/g). A similar trend can be observed with Marion sludge (Table 13.3). At the low addition rates (2 to 20 mg/g) 66 to 94% of the added Cd was retained, whereas only 30 to 53% of the added Cd was retained at the higher concentrations (30 to 50 mg/g).

Tables 13.1, 13.2 and 13.3 show that the proportion of retained Cd which was extractable with $Ca(NO_3)_2$ solution increased with increasing Cd addition rates from 0 to 50 mg/g. The proportional increase in $Ca(NO_3)_2$-extractable Cd ranged over the range in metal addition rates from 0.6 to 21.7%, 1.8 to 18.0% and 1.7 to 38.0% for Frankfort, Anderson and Marion sludges, respectively. This finding suggests that, at the higher Cd loading rates (30 to 50 mg/g) Cd is loosely bound in the sludge, possibly by weak interactions (exchange reactions) with sludge components.

The proportion of added Cd retained by Frankfort, Anderson and Marion sludges which was extractable by DTPA decreased with increasing Cd addition rate. Approximately 49% of the Cd retained by Frankfort sludge was extractable with DTPA at the 2 mg/g Cd addition rate. Extraction of retained Cd decreased to 15% at the 50 mg/g Cd addition for Frankfort sludge. A similar trend can be observed with Anderson sludge. Approximately 43% of the retained Cd was extractable with DTPA at the 2 mg/g addition level, whereas only 19% was extractable by DTPA at the 50 mg/g addition rate. Marion sludge exhibited similar behavior. A decrease of 13% in the proportion of retained Cd which was extractable with DTPA occurred over the Cd addition range 2 to 50 mg/g. At a solution to solids ratio of 10:1 the maximum amount of Cd chelated by DTPA extract is 11,200 μg Cd/g sludge (this assumes Cd is the only metal interacting with DTPA). The relatively low amount of Cd chelated by DTPA in Cd-treated Frankfort, Anderson and Marion sludges, suggests that Ca, Mn, Fe, Al and other divalent metal ions were also being chelated by DTPA. This would cause a decrease in the proportion of sludge-Cd extractable with DTPA.

Tables 13.1, 13.2 and 13.3 show that the proportion of added Cd retained by Frankfort, Anderson and Marion sludges which was extractable with HNO_3 increased with increasing Cd concentrations. Therefore, an inverse relationship exists in the proportion of retained Cd which is extractable with DTPA and HNO_3. Approximately 32% of retained Cd was extractable with HNO_3 at the 2 mg/g Cd addition rate, whereas 61% of the retained Cd was extractable at the 50 mg/g addition level in Frankfort sludge. Similar trends can be observed with Anderson and Marion sludges. Approximately 39% of the retained Cd was extractable with HNO_3 at the 2 mg/g addition rate which increased to 48% at the 50 mg/g rate with the Anderson sludge. Marion sludge exhibited a 25% extraction of retained Cd at the 2 mg/g rate which increased to 57% at the 50 mg/g Cd addition rate.

The recovery of retained Cd by $Ca(NO_3)_2$, DTPA, and HNO_3 resulting from sequential extraction of Frankfort, Anderson and Marion sludges, ranged from 80 to 100%, 85 to 95% and 75 to 108%, respectively (Tables 13.1, 13.2 and 13.3). The large proportion of retained Cd extractable with $Ca(NO_3)_2$ and DTPA suggests that Cd is either loosely bound in sludge by exchange reactions or is present as a chelate with organic matter or precipitated as a carbonate. Cadmium in chelates and carbonate precipitates is solubilized in DTPA reagent.

The pH values of the Cd solutions were determined after equilibration with Frankfort, Anderson and Marion sludges. The equilibrium pH values of Frankfort, Anderson and Marion sludges are presented in Tables 13.1, 13.2 and 13.3. With the solution containing the highest Cd concentration (2.5 mg/ml), there was a significant increase in solution pH from 4.7 initially to 6.5 after equilibration with Frankfort sludge. Similarly, with the 0.1 mg/ml Cd solution the pH increased from 5.7 initially to 7.2 after equilibration. Similar trends were observed with the Anderson and Marion sludges. This finding suggests that Cd retention by calcareous sludges may be due at least partially to precipitation as carbonates resulting from an increase in solution pH during interaction of the metal with sludges. The soluble Cd concentration as a function of pH was determined in the absence of sludge. As pH increases in the range 4.7 to 11.1 a significant decrease in soluble Cd concentration was observed. This suggests that Cd precipitation as a carbonate may account for much of the retention observed during the equilibration of calcareous sludges with Cd solutions. Precipitation of Cd in sludge as hydroxides is unlikely because Patterson _et al._ (1977) showed that solution pH values of 12 or greater are required for $Cd(OH)_2$ formation. The results implied that non-precipitation mechanisms, such as complexation by organic matter and sorption by amorphous Fe and Al, may also occur during reaction of Cd with sludge. These non-precipitation mechanisms may account for the retention of 50% or more of the added Cd.

Retention of Metals by Untreated Acidic Sludges

It has been shown that calcareous sludges have the capacity to retain added metals. This metal retention is primarily pH dependent. A reduction in the proportion of added metals retained was observed at solution pH values at or below pH 5.5. Approximately 50% of the metal retained is due primarily to non-precipitation mechanisms at high metal addition rates. The following discussion is concerned with a comparison of the retention of Cd, Cu, Zn and Ni by a calcareous (pH 7.2) sludge and two acidic sludges (pH 5.5 and 4.5).

Table 13.9 presents the retention of Cd by Frankfort sludge (pH 7.2), West Lafayette sludge (pH 5.5) and acidified Frankfort sludge (pH 4.5). Approximately 95% of the added Cd was retained by Frankfort sludge at the 4 mg/g Cd addition rate and 63% at the 50 mg/g Cd addition rate. West Lafayette sludge retained 75% of the added Cd at the 4 mg/g Cd addition rate and 49% at the highest addition rate. Acidified Frankfort sludge retained 69% of the added Cd at the 4 mg/g Cd addition rate and only 3% at the 50 mg/g Cd addition rate.

Table 13.9 shows that the proportion of the retained Cd which was extractable with $Ca(NO_3)_2$ solution increased with increasing Cd concentration for all three sludges. Acidified Frankfort sludge exhibited the largest increases in the proportion of retained Cd which was extracted with $Ca(NO_3)_2$. This finding suggests that Cd is more loosely bound to low pH sludges as compared to calcareous sludges. It appears that the proportion of Cd extractable with $Ca(NO_3)_2$ is inversely related to pH. At the 50 mg/g Cd addition rate the acidified Frankfort sludge equilibrium pH is 4.3. Cadmium is readily desorbed from the sludge surfaces when high amounts of Cd are retained and when sludge pH values are low. The finding that a significant proportion of native Cd in acidified sludge is extractable with $Ca(NO_3)_2$ is consistent with soluble Cd-pH relationships for sorbed or precipitated Cd. This supports the finding that 42.5% of the retained Cd was extracted by $Ca(NO_3)_2$ at the 50 mg/g Cd addition rate. Frankfort and West Lafayette sludges exhibited increases in the proportion of retained Cd extractable with $Ca(NO_3)_2$ as the rate of metal addition increased. These findings support the finding that at low pH, sorbed Cd can be extracted with $Ca(NO_3)_2$, whereas, at intermediate pH values (5.3 to 6.9), Cd retained in the sludge is not extractable by $Ca(NO_3)_2$.

The proportion of added Cd retained by Frankfort, West Lafayette and acidified Frankfort sludges which was extractable by DTPA is presented in Table 13.9. As the Cd addition rates increase from 4 to 50 mg/g the proportion of retained Cd in Frankfort and West Lafayette sludges extractable with DTPA decreased from 49 and 88%, respectively, at the 4 mg/g Cd addition rate to 15 and 18%, respectively, at the 50 mg/g Cd addition rate. Acidified Frankfort sludge also exhibited a decrease in the proportion of Cd retained which was extracted by DTPA as the metal addition rate increased from 4 to 20 mg/g, but increased markedly at the 50 mg/g Cd addition rate. This finding suggests that at the low sludge pH values induced by the 50 mg/g addition rate, DTPA is extracting the Cd weakly bound by organic matter and amorphous Fe and Al complexes. The only Cd ions unextractable with DTPA at pH 4.3 would be the occluded Cd. Maximum saturation of DTPA with added Cd did not occur. At a solution to solids ratio of 10:1 the maximum amount of Cd which can be chelated by DTPA is 11,200 μg Cd/g sludge (this assumes that Cd is the only metal interacting with DTPA). This suggests that if additional DTPA was added to the Cd-sludge solution additional Cd could be extracted. Table 13.9 shows that the proportion of retained Cd which was extractable with HNO_3 increased with increasing Cd addition rates. Therefore, an inverse relationship exists between the proportion of retained Cd extractable with DTPA and HNO_3. It is likely that HNO_3 is removing the occluded Cd and co-precipitated Cd in acidified Frankfort sludge.

The recovery of added Cd resulting from sequential extraction of $Ca(NO_3)_2$, DTPA and HNO_3 Frankfort, West Lafayette and acidified Frankfort sludges are also presented in Table 13.9. Approximately 78 to 99% of the added Cd was recovered from Frankfort sludge. Recoveries of Cd from West Lafayette and acidified Frankfort sludges were 70 to 137 and 68 to 123%, respectively. The large variation in the recoveries of Cd from West Lafayette and acidified Frankfort sludges may be partially due to the wide variation in pH following treatment with metal solutions.

The pH values of the Cd solutions were determined before and after equilibration with Frankfort, West Lafayette and acidified Frankfort sludges, and are presented in Table 13.9. There was a significant increase in solution pH after equilibration of Cd solutions with Frankfort sludge. This finding suggests that Cd retention by calcareous sludges may be due partially to precipitation as carbonates resulting from an increase in pH.

The pH values of metal solutions after equilibrium with acidified Frankfort sludge were less than 5.4. West Lafayette sludge exhibited intermediate Cd retention characteristics and intermediate pH values (5.3 to 6.4) in the equilibrium solution. It was observed at pH 6.4, the maximum pH value in the Cd solution-West Lafayette sludge mixtures, that over 75% of the Cd was in solution. This finding suggests that non-precipitation mechanisms as well as precipitation reactions are involved in the retention of Cd by West Lafayette sludge. Similar conclusions can be drawn for the retention of Cd with acidified Frankfort sludge. At pH values between 4.8 and 5.4 over 90% of the Cd is still in solution. This suggests that at the equilibrium pH values for the Cd solution-acidified Frankfort sludge mixtures, approximately 90% of the Cd retained may be due to non-precipitation type mechanisms. The equilibrium pH value for the 50 mg/g Cd solution-acidified Frankfort sludge mixture was 4.3. This suggests that at the 50 mg/g Cd addition rate retention of Cd by acidified Frankfort sludge was due primarily to non-precipitation type mechanisms.

Removal of Indigenous Metals by Various Extractants

It appears that sludge consists of a set of heterogenous components capable of complexing varying amounts of Cd, Cu, Pb, Ni and Zn. Stover *et al*. (1976) used a sequential extraction procedure to characterize metals in sewage sludges. Sorbed metals were removed with 0.5 *M* KF, organically-bound with 0.1 *M* $Na_4P_2O_7$, carbonate-bound with 1 *M* EDTA, and sulfide-bound with 1 *M* HNO_3. In the present study organically-bound metals are removed from sewage sludge with H_2O_2 and 0.1 *M* $Na_4P_2O_7$, carbonate-bound metals with 1 *M* sodium acetate, sorbed metals with NH_4F, and citrate-dithionite-bicarbonate reagent was used to remove metals associated with iron and aluminum hydrous oxides. No sequential extractions were used.

Table 13.14 presents the amounts of metal removed from unamended Anderson sludge by various extractants. Approxiamtely 71% of the native Cu and 45% of the Pb present in Anderson sludge was extracted with $Na_4P_2O_7$. The NaOAc treatment removed 89% of the Cd present in Anderson sludge and 100% of the Zn present. The CDB extraction removed the highest proportion of Ni.

Table 13.15 presents the concentrations of metals removed from unamended Frankfort sludge by various extractants. Approximately 69% of the native Cu present in Frankfort sludge was extracted with $Na_4P_2O_7$. The NaOAc treatment removed 96, 99 and 115% of the native Cd, Zn and Ni present in Frankfort sludge, respectively. The highest amount of Pb was also associated with the NaOAc extraction. The amount of Ni extracted with CDB was essentially equal to the amount extracted with NaOAc. Approximately 5% of the native Cu was extracted from West Lafayette sludge with CDB (Table 13.16). The amounts of Cd, Zn, Ni and Pb extracted with CDB were 23, 50, 62 and 15%, respectively.

The results obtained from studies of metal removal from unamended sludges suggest three sludge components were involved in complexing metals during sludge digestions: (1) organic matter, (2) carbonates and (3) amorphous Fe, Al and Mn hydrous oxides. Copper was primarily removed from Anderson and Frankfort sludges by the $Na_4P_2O_7$ treatment. This finding suggests that Cu may be primarily organically bound in sludge. The treatment of Anderson and Frankfort sludges with NaOAc resulted in the highest amounts of Cd and Zn in the extract. This finding suggests that Cd and Zn are largely associated with the carbonate fraction of sludges because NaOAc should not affect any other sludge component. The finding that a large amount of Ni was removed during the CDB extraction of Anderson, Frankfort and West Lafayette sludges suggests that Ni is complexed by amorphous Fe, Al and Mn hydrous oxides in sludges. Nickel was also removed in high amounts of NaOAc extraction of Frankfort sludge. This finding suggests that Ni may also be associated with the carbonate components of sludges in addition to amorphous hydrous oxides. The $Na_4P_2O_7$ extraction of Anderson sludge and the NaOAc treatment of Frankfort sludge resulted in the highest amounts of Pb solubilized. This finding suggests two sludge components may be involved in Pb complexation-organic matter and carbonates. The treatment of Anderson and Frankfort sludges with H_2O_2 resulted in the lowest amounts of Cu, Cd, Zn, Ni and Pb extracted. This finding was not expected because H_2O_2 treatment under acidic conditions removes both carbonates and organic matter. One possible explanation for the finding is that metals released during decomposition of organic matter and carbonate may be precipitated as phosphates or other insoluble compounds.

Sites of Metal Retention in Sludge

The results obtained from the retention of added Cd, Cu, Ni and Zn by Anderson, Frankfort, Marion and West Lafayette sludges demonstrate that sludges have the capacity to sorb and retain added metals. This suggests that sludges, like soils, contain a wide variety of sites capable of metal retention. Retention mechanisms that may be operative include: complexation, chelation, exchange reactions and precipitation. The following discussion summarizes data obtained to identify sludge components responsible for metal retention.

Figure 13.1 presents the retention of added Cd by Anderson whole sludge and NH_4F-, $Na_4P_2O_7$-, CDB-, NaOAc-, or H_2O_2-extracted Anderson sludge. The retention of added Cd was highest with the Anderson whole sludge and NH_4F- or $Na_4P_2O_7$-extracted sludge and lowest with NaOAc-, CDB- or H_2O_2-extracted sludge. The proportion of added Cd retained by Anderson sludge was found to decrease in the following order: NH_4F extracted > whole sludge > $Na_4P_2O_7$ extracted > NaOAc extracted > CDB extracted > H_2O_2 treated. Retention of added Cd by NH_4F-, or $Na_4P_2O_7$-extracted Anderson sludge and the whole sludge was linear over the 5 to 20 mg/g addition range. The CDB-, NaOAc- or H_2O_2-extracted Anderson sludge exhibited a curvilinear relationship to added Cd. A leveling off in Cd retention by the H_2O_2-extracted Anderson sludge was evident at metal rates higher than 10 mg/g.

Figure 13.2 presents the retention of added Cd by Frankfort whole sludge, $Na_4P_2O_7$-, CDB-, H_2O_2- or NaOAc-extracted Frankfort sludge. The retention of added Cd was highest with the whole sludge and $Na_4P_2O_7$-extracted sludge. The ability of the $Na_4P_2O_7$ extracted Frankfort sludge to retain more Cd than the whole sludge may be due to the precipitation of $Na_4P_2O_7$ as $Ca_2P_2O_7$, thereby creating more sites for Cd retention. The CDB-, H_2O_2- and NaOAc-extracted Frankfort sludges exhibited the lowest Cd retention capacities. The retention of added Cd by Frankfort sludge was found to decrease in the following order: $Na_4P_2O_7$ extracted > whole sludge > CDB extracted > H_2O_2 extracted=NaOAc extracted. Retention of added Cd by $Na_4P_2O_7$-extracted Frankfort sludge and whole sludge was linear over the 5 to 20 mg/g addition range. The CDB-, NaOAc- and H_2O_2 extracted sludge did not exhibit a linear relationship to added Cd. A rapid decrease in Cd retention by the H_2O_2- and NaOAc-extracted Frankfort sludge occurred at added metal concentrations above 10 mg/g.

Figure 13.3 presents the retention of added Cd by West Lafayette sludge and CDB-extracted West Lafayette sludge. The retention of added Cd was highest with the CDB extracted West Lafayette sludge and lowest with the West Lafayette whole sludge. Retention of added Cd by the CDB-extracted West Lafayette sludge was linear over the 5 to 20 mg/g range.

Figures 13.1 and 13.2 show that there is a large decrease in added Cd retention following the extraction of Anderson and Frankfort sludges with CDB, NaOAc, or H_2O_2. This finding suggests that three major sludge components may be involved in Cd retention: (1) amorphous Fe, Al and Mn hydrous oxides, (2) carbonates and (3) organic matter. The drop in Cd retention (as compared to whole sludge) by CDB-extracted Anderson and Frankfort sludges can be attributed to the removal of amorphous Fe, Al and Mn hydrous oxides. The lower retention capacity of Frankfort sludge (as compared to Anderson sludge) for Cd following extraction with CDB may be partially explained by the higher Fe content of the CDB extract. Table 13.15 shows that approxiamtely 61% of the native Fe was extracted from Frankfort sludge with CDB as compared to 53% in Anderson sludge (Table 13.14). This finding suggests greater removal of amorphous hydrous oxides from Frankfort sludge by CDB. In addition to the removal of Fe, Al and Mn hydrous oxides by CDB, some carbonates were also destroyed. Apprximately 11 and 55% of the Ca present in Anderson and Frankfort sludges, respectively, was removed by treatment with CDB.

Extraction of Anderson and Frankfort sludges with NaOAc resulted in a further decrease (as compared to CDB-extracted sludges) in the retention capacity of these sludges for Cd (Figures 13.1 and 13.2). This finding suggests that carbonates are involved in Cd retention in Anderson and Frankfort sludges. As compared to Frankfort sludge, a smaller decrease in Cd retention following NaOAc extraction of Anderson sludge was observed. This finding may be partially related to the higher removal of Ca by NaOAc extraction of Frankfort sludge (as compared to Anderson sludge), suggesting more efficient carbonate removal with Frankfort sludge. Approxiamtely 55% of the native Ca was extractable by NaOAc with Frankfort sludge and only 11% with Anderson sludge (Tables 13.14 and 13.15).

Oxidation of organic matter by H_2O_2 resulted in an additional large decrease (as compared to NaOAc-extracted sludge) in Cd retention by Anderson sludge (Figure 13.1). This finding suggests that organic matter is a primary site for Cd retention in Anderson sludge. The ability of soil organic matter to form complexes with metal ions has been well established. Therefore, it can be assumed that these soil organic matter-metal reactions are also operative with sludge organic matter because of their similar properties. The high Ca content of the H_2O_2 extract of Anderson sludge suggests that carbonates were also destroyed during the oxidation of organic matter. However, the decrease in Cd retention induced by H_2O_2 treatment (beyond the NaOAc carboante removal) is attributed largely to destruction of organic matter and elimination of numerous metal binding sites. Similarly, for Frankfort sludge a drop in Cd retention was observed following treatment with H_2O_2. This finding suggests that organic matter is involved in Cd retention by Frankfort sludge.

The higher retention capacity of the CDB extracted West Lafayette sludge (as compared to untreated sludge) may be partially due to its low Fe extractable fraction. Approxiamtely 5% of the native Fe was extracted by CDB in the West Lafayette sludge as compared to 53 and 61% for Anderson and Frankfort sludges, respectively (Tables 13.14 to 13.16). Therefore, CDB treatment would not be expected to reduce Cd retention because the extraction was ineffective in removing amorphous hydrous oxides.

With Anderson sludge the majority of the Cd is retained by carbonates and organic matter components. Carbonate appears to be of primary importance in Cd retention by Frankfort sludge, whereas Fe, Al, and Mn hydrous oxides and organic matter seem to be of secondary importance.

Table 13.17 presents the equilibrium pH values of the extracted Anderson, Frankfort and West Lafayette sludges-Cd solution mixtures. A decrease in pH with increasing Cd concentration can be observed for sludge treated with each extractant. The equilibrium pH values of the NH_4F and $Na_4P_2O_7$ extracted Anderson sludge-Cd mixtures are high (> 6.6), suggesting that added Cd may be precipitating as insoluble carbonate. This would lead to the appearance of high Cd retention by NH_4F- and $na_4P_2O_7$-extracted sludges as was found. The ineffectiveness of NH_4F and $Na_4P_2O_7$ treatments in reducing Cd retention by sludges may in fact be related to the high sludge pH values after extraction. However, the high equilibrium pH values of the CDB-extracted Anderson and Frankfort sludges-Cd solution mixtures did not lead to increased retention of added Cd. This finding suggests that more than solution pH is involved in metal retention by sludges. The low equilibrium pH values for the NaOAc extracted sludge-Cd solution mixtures are due to the absence of carbonates. A decrease in $CaCO_3$ content corresponds to an increase in Cd solubility. No precipitation of $CdCO_3$ would be extracted with NaOAc treated sludges amended with Cd solution because of low pH and carbonate content.

Table 13.21 presents the percentage of added metal retained when the metal is added at the rate of 10 mg/g to unextracted and extracted sludge. Retention of added Cd was lowest with the H_2O_2-extracted Anderson and Frankfort sludges, and was somewhat higher with the NaOAc- and CDB-extracted Anderson and Frankfort sludges. Retention of Cu by Anderson sludge was lowest with the H_2O_2-extracted sludge, followed by CDB- and NaOAc-extracted Anderson sludges. Frankfort sludge exhibited 4% retention of added Cu following the CDB extraction, 21% after the H_2O_2-extraction and 32% following carbonate removal by NaOAc. Retention of Ni by the CDB extracted Frankfort and West Lafayette sludges was zero at the 10 mg/g Ni addition rate. Anderson sludge exhibited zero retention of added Ni following the oxidation of organic matter by H_2O_2. Retention of added Zn at the 10 mg/g Zn addition rate was lowest with the H_2O_2-extracted Anderson and Frankfort sludges and was somewhat higher with CDB- and NaOAc-extracted sludges.

The preceding discussion suggests that retention of added Cd, Cu, Ni and Zn by sludges is primarily due to complexation and chelation by organic matter (humic materials), complexation by amorphous Fe, Al and Mn hydous oxides and precipitation by carbonates.

In an attempt to generalize the findings concerning the components responsible for metal retention in sludges the following order of importance can be assigned for each metal:

Cadmium: carbonate > organic matter > hydrous oxides
Copper: carbonate > organic matter = hydrous oxides
Nickel: carbonate > organic matter = hydrous oxides
Zinc: carbonate > organic matter > hydrous oxides

Table 13.1. Retention of added cadmium by air-dried Frankfort sludge.

Cadmium added	Equilibrium pH*	Added Cd unretained	Retained Cd extracted by:			Recovery of added cadmium
			$Ca(NO_3)_2$	DTPA	HNO_3	
mg/g		µg/g				%
0	7.2	0	5	656	1009	--
2	7.3	69	17	1594	1619	90
3	7.2	129	39	1933	2080	90
4	7.4	206	54	2706	2430	95
5	7.7	274	112	3330	2721	96
10	6.9	1013	262	3856	5389	90
20	7.0	2894	1080	3756	9062	78
30	6.9	7489	2083	3609	12493	81
40	7.0	13887	4010	4438	14795	89
50	6.9	23623	5726	4486	16987	99

* pH of sludge - 0.01 M $Ca(NO_3)_2$ - cadmium solution after shaking for 24 hours.

Table 13.2. Retention of added cadmium by air-dried Anderson sludge.

Cadmium added	Equilibrium pH*	Added Cd unretained	Retained Cd extracted by:			Recovery of added cadmium
			$Ca(NO_3)_2$	DTPA	HNO_3	
mg/g		µg/g				%
0	7.2	0	7	126	178	--
2	7.3	97	42	946	912	87
3	7.0	175	74	1267	1335	86
4	7.3	248	116	1482	1834	85
5	6.9	407	165	1667	2265	85
10	7.0	1484	482	2265	5078	90
20	7.2	5524	1416	3064	7033	84
30	6.6	12516	2472	3543	9902	94
40	6.1	21038	3197	3859	9118	92
50	6.1	28942	3797	4111	10187	94

* pH of sludge - 0.01 M $Ca(NO_3)_2$ - cadmium solution after shaking for 24 hours.

Table 13.3. Retention of added cadmium by air-dried Marion sludge.

Cadmium added	Equilibrium pH*	Added Cd unretained	Retained Cd extracted by: $Ca(NO_3)_2$	DTPA	HNO_3	Recovery of added cadmium
mg/g		----------	µg/g	----------		%
0	7.2	0	20	93	379	-
2	7.0	114	53	872	859	76
3	7.0	210	87	1344	929	74
4	7.9	460	112	1803	1329	80
5	7.0	487	151	2278	1520	81
10	7.4	2118	433	3823	3403	93
20	6.7	6729	1266	4878	5532	90
30	6.5	14207	2362	4437	5312	86
40	6.9	24464	3962	4585	7972	101
50	6.6	34929	5732	5271	8892	108

* pH of sludge - 0.01 M $Ca(NO_3)_2$ - cadmium solution after shaking for 24 hours.

Table 13.4. Retention of added copper by air-dried Frankfort sludge.

Copper added	Equilibrium pH*	Added Cu unretained	Retained Cu extracted by:			Recovery of added copper
			$Ca(NO_3)_2$	DTPA	HNO_3	
mg/g		---------- µg/g ----------				%
0	7.2	0	144	450	909	--
2	8.1	259	144	1102	1719	92
3	7.3	225	144	1419	2165	88
4	7.3	240	144	1430	2642	82
5	7.5	244	144	1852	3257	85
10	7.5	406	192	2712	5997	81
20	6.3	2967	454	4044	12130	91
30	6.0	7873	1050	4300	16191	96
40	5.8	16220	2124	4873	15871	94
50	5.6	23147	3358	5103	16366	93

* pH of sludge - 0.01 M $Ca(NO_3)_2$ - copper solution after shaking for 24 hours.

Table 13.5. Retention of added copper by air-dried Marion sludge.

Copper added	Equilibrium pH*	Added Cu unretained	Retained Cu extracted by: $Ca(NO_3)_2$	DTPA	HNO_3	Recovery of added copper
mg/g		µg/g				%
0	7.2	0	111	546	851	-
2	7.3	282	116	1687	1502	102
3	7.5	354	106	2086	1865	98
4	7.3	387	116	2556	2340	98
5	7.3	478	130	2593	2681	90
10	7.1	802	192	4259	5275	92
20	6.2	5729	811	5988	8874	100
30	6.0	13643	1622	5440	9795	97
40	5.7	20997	2674	6297	9436	95
50	5.6	34012	3631	6271	10000	105

* pH of sludge - 0.01 M $Ca(NO_3)_2$ - copper solution after shaking for 24 hours.

Table 13.6. Retention of added copper by air-dried Anderson sludge.

Copper added	Equilibrium pH*	Added Cu unretained	Retained Cu extracted by:			Recovery of added copper
			$Ca(NO_3)_2$	DTPA	HNO_3	
mg/g		------------------	µg/g	------------------		%
0	7.2	0	211	1248	202	-
2	7.5	373	178	1626	2964	94
3	7.3	478	173	1965	3375	93
4	7.3	335	197	2548	3694	91
5	7.4	521	173	2949	4828	100
10	6.2	1144	240	3954	7403	92
20	5.8	5967	528	4864	12229	101
30	5.5	14549	1517	4616	12592	99
40	5.4	22573	2100	4846	13011	98
50	5.5	31329	3138	5464	13905	101

* pH of sludge - 0.01 M $Ca(NO_3)_2$ - copper solution after shaking for 24 hours.

Table 13.7. Retention of added zinc by air-dried Frankfort sludge.

Zinc added	Equilibrium pH*	Added Zn unretained	Retained Zn extracted by: $Ca(NO_3)_2$	DTPA	HNO_3	Recovery of added zinc
mg/g		µg/g				%
0	7.2	0	7	820	1180	-
2	7.3	113	33	1312	2120	89
3	7.2	249	74	1412	2609	87
4	7.4	400	120	1491	3335	89
5	6.9	590	180	1499	3863	88
10	6.5	2310	645	1552	5981	87
20	6.4	7300	1720	1428	7619	82
30	6.3	13400	2640	1436	8150	80
40	6.1	22000	3640	1288	9931	88
50	5.7	30000	4720	1136	10164	89

* pH of sludge - 0.01 M $Ca(NO_3)_2$ - zinc solution after shaking for 24 hours.

Table 13.8. Retention of added nickel by air-dried Frankfort sludge.

Nickel added	Equilibrium pH*	Added Ni unretained	Retained Zn extracted by:			Recovery of added nickel
			$Ca(NO_3)_2$	DTPA	HNO_3	
mg/g		µg/g				%
0	7.2	0	92	175	298	-
2	7.3	689	158	871	689	94
3	7.4	1100	229	1177	885	95
4	7.5	1517	336	1456	941	93
5	7.5	1740	451	1700	1380	95
10	7.4	4139	478	2619	1786	86
20	7.3	8013	930	3361	1699	68
30	6.8	13474	1490	3778	2183	69
40	6.3	22606	1893	3899	2705	77
50	5.9	31697	2190	4266	3263	82

* pH of sludge - 0.01 M $Ca(NO_3)_2$ - nickel solution after shaking for 24 hours.

Table 13.9. Retention of added cadmium by air-dried Frankfort sludge (pH 7.2), West Lafayette acid sludge (pH 5.5) and Frankfort acid sludge (pH 5.5).

Type of sludge	Cadmium added	Equilibrium pH*	Added Cd unretained	Retained cadmium extracted by:			Recovery of added cadmium
				$Ca(NO_3)_2$	DTPA	HNO_3	
	mg/g		µg/g				%
Frankfort pH 7.2	0	7.2	0	5	656	1009	-
	4	7.4	206	54	2706	2430	95
	10	6.9	1013	262	3856	5389	90
	20	7.0	2894	1080	3756	9062	78
	50	6.9	23623	5726	4486	16987	99
West Lafayette pH 5.5	0	5.6	0	98	0	0	-
	4	6.4	1017	390	2622	1597	137
	10	6.0	1651	653	2496	2218	70
	20	5.7	5188	1850	4156	7378	92
	50	5.3	25750	5088	4269	13248	97
Frankfort pH 4.5	0	4.5	0	99	63	158	-
	4	5.4	1242	144	1546	1268	97
	10	4.8	2416	747	2335	1540	68
	20	4.6	8547	2797	2788	2072	80
	50	4.3	48726	5520	3407	4121	123

* pH of sludge - 0.01 M $Ca(NO_3)_2$ - cadmium solution after shaking for 24 hours.

Table 13.10. Retention of added copper by air-dried Frankfort sludge (pH 7.2), West Lafayette acid sludge (pH 5.5) and Frankfort acid sludge (pH 4.5).

Type of sludge	Copper added	Equilibrium pH*	Added Cu unretained	Retained cadmium extracted by:			Recovery of added copper
				$Ca(NO_3)_2$	DTPA	HNO_3	
	mg/g		µg/g	µg/g	µg/g	µg/g	%
Frankfort pH 7.2	0	7.2	0	144	450	909	-
	4	7.3	240	144	1430	2642	82
	10	7.5	406	192	2712	5997	81
	20	6.3	2967	454	4044	12130	91
	50	5.6	23147	3358	5103	16366	93
West Lafayette pH 5.5	0	5.6	0	57	44	50	-
	4	5.9	1141	145	1223	1502	97
	10	5.6	1371	163	3411	3068	79
	20	5.4	1649	230	4126	8790	73
	50	4.0	22363	3121	4478	18356	76
Frankfort pH 4.5	0	4.5	0	0	224	492	-
	4	6.3	718	0	1994	2336	107
	10	5.1	3390	603	2935	4295	105
	20	4.4	13905	1726	3406	6104	121
	50	4.1	50036	3849	3703	7252	128

* pH sludge - 0.01 M $Ca(NO_3)_2$ - copper solution after shaking for 24 hours.

Table 13.11. Retention of added nickel by air-dried Frankfort sludge (pH 7.2), West Lafayette acid sludge (pH 5.5) and Frankfort acid sludge (pH 4.5).

Type of sludge	Nickel added	Equilibrium pH*	Added Ni unretained	Retained cadmium extracted by: $Ca(NO_3)_2$	DTPA	HNO_3	Recovery of added nickel
	mg/g		µg/g				%
Frankfort pH 7.2	0	7.2	0	92	175	298	-
	4	7.5	1517	336	1456	941	93
	10	7.4	4139	478	2619	1786	86
	20	7.3	8013	930	3361	1699	68
	50	5.9	31697	2190	4266	3263	82
West Lafayette pH 5.5	0	5.6	0	45	10	23	-
	4	6.0	1843	1021	674	207	92
	10	5.8	4787	2165	1152	320	84
	20	5.6	13784	4150	1994	531	102
	50	5.3	43508	6756	2429	587	106
Frankfort pH 4.5	0	4.5	0	28	1	0	-
	4	7.0	2025	550	106	85	69
	10	5.8	6973	1169	363	48	85
	20	5.5	16420	2491	898	12	99
	50	5.3	56758	6588	1743	0	130

* pH of sludge - 0.01 M $Ca(NO_3)_2$ - nickel solution after shaking for 24 hours.

Table 13.12. Retention of added zinc by air-dried Frankfort sludge (pH 7.2), West Lafayette acid sludge (pH 5.5) and Frankfort acid sludge (pH 4.5).

Type of sludge	Zinc added	Equilibrium pH*	Added Zn unretained	Retained cadmium extracted by:			Recovery of added zinc
				$Ca(NO_3)_2$	DTPA	HNO_3	
	mg/g		------------------	µg/g	------------------		%
Frankfort pH 7.2	0	7.2	0	7	820	1180	-
	4	7.4	400	120	1491	3335	89
	10	6.5	2310	645	1552	5981	87
	20	6.4	7300	1720	1428	7619	82
	30	5.7	30000	4720	1136	10164	89
West Lafayette pH 5.5	0	5.6	0	78	385	575	-
	4	6.5	1498	239	2545	1875	122
	10	5.7	2345	213	3223	6123	108
	20	5.6	9005	403	3421	14087	128
	50	5.0	14498	2390	3503	19030	77
Frankfort pH 4.5	0	4.5	0	30	0	0	-
	4	6.4	599	365	2132	0	77
	10	5.0	5551	1364	1712	13	86
	20	4.9	16316	2299	3423	191	110
	50	4.6	35924	3233	7159	820	94

* pH of sludge - 0.01 M $Ca(NO_3)_2$ - zinc solution after shaking for 24 hours.

Table 13.13. Comparison of metal retention by the West Lafayette (pH 5.5) and Frankfort (pH 4.5) acid sludges with the calcareous Anderson, Frankfort and Marion sludges at the 10 mg of metal per gram of sludge rate.

Metal added	Source of sludge				
	Anderson	Frankfort	Marion	Frankfort (acid)	West Lafayette (acid)
	% of added metal retained				
Cadmium	85	90	79	76	83
Copper	89	96	92	66	86
Nickel	61	59	--	30	52
Zinc	74	77	--	45	77

Table 13.14. Metals removed from unamended Anderson sewage sludge by various extractants.

Extractant	Metals in Anderson sludge								
	Cu	Cd	Zn	Ni	Pb	Fe	Al	Ca	Mn
	μg/g								
HNO_3-$HClO_4$ digestion*	3359	283	6546	1857	923	16043	997	49103	181
H_2O_2 (30%)	24	20	148	150	0	16	0	12634	25
NaOAc (1 $\underline{M}$)	992	253	7160	1300	389	0	725	13311	88
$Na_4P_2O_7$ (0.1 $\underline{M}$)	2380	97	5476	629	412	4089	1808	933	69
Na Citrate (0.3 $\underline{M}$)-$NaHCO_3$ (1 $\underline{M}$) -$Na_2S_2O_7$ (0.12 $\underline{M}$)	158	41	2505	2874	54	8540	1835	5183	106
NH_4F (0.5 $\underline{M}$)	1782	5	653	272	4	73	653	2	0

* Total metal concentration.

Table 13.15. Metals removed from unamended Frankfort sewage sludge by various extractants.

Extractant	Metals in Frankfort sludge								
	Cu	Cd	Zn	Ni	Pb	Fe	Al	Ca	Mn
	µg/g								
HNO_3-$HClO_4$ digestion*	939	1370	1979	424	529	49418	5311	36963	330
H_2O_2 (30%)	4	111	99	84	6	0	470	9764	19
NaOAc (1 M)	188	1318	1954	487	2763	1967	72	29141	154
$Na_4P_2O^7$ (0.1 M)	646	531	1656	219	957	9118	774	777	73
Na Citrate (0.3 M)-$NaHCO_3$ (1 M) -$Na_2S_2O_7$ (0.12 M)	169	420	1112	441	631	30360	1119	20047	149

* Total metal concentration.

Table 13.16. Metals removed from unamended West Lafayette sewage sludge by CDB.

Extractant	Metals in West Lafayette sludge								
	Cu	Cd	Zn	Ni	Pb	Fe	Al	Ca	Mn
	µg/g								
HNO_3-$HClO_4$ digestion*	796	13	867	93	268	6876	2652	30668	116
Na Citrate (0.3 M)-$NaHCO_3$ (1 M) -$Na_2S_2O_7$ (0.12 M)	42	3	437	58	40	329	1329	12258	114

* Total metal concentration.

Table 13.17. The pH values of 0.01 M $Ca(NO_3)_2$- cadmium solutions after equilibration with Anderson, Frankfort and West Lafayette sludges which had been extracted with reagents used to remove specific components.

Type of sludge	Cadmium added	Extractants used to remove specific sludge components				
		NH_4F	$Na_4P_2O_7$	Na Citrate - $NaHCO_3$ - $Na_2S_2O_4$	NaOAc	H_2O_2
	mg/g	pH*				
Anderson	5	8.2	7.7	7.8	5.5	6.1
	10	8.2	8.2	7.6	5.4	6.1
	20	7.8	5.0	7.2	5.2	5.8
Frankfort	5	---	6.9	7.6	4.7	6.0
	10	---	6.6	7.8	4.6	6.0
	20	---	6.2	7.9	4.6	5.8
West Lafayette	4	---	---	6.5	---	---
	20	---	---	6.5	---	---
	50	---	---	5.7	---	---

* pH of 0.01 M $Ca(NO_3)_2$ + Cd solution after equilibration with sludge previously extracted with reagents shown.

Table 13.18. The pH values of 0.01 M $Ca(NO_3)_2$ - copper solutions after equilibration with Anderson, Frankfort and West Lafayette sludges which had been extracted with reagents used to remove specific components.

Type of sludge	Copper added	Extractants used to remove specific sludge components				
		NH_4F	$Na_4P_2O_7$	Na Citrate - $NaHCO_3$ - $Na_2S_2O_4$	NaOAc	H_2O_2
	mg/g	pH*				
Anderson	5	7.8	7.3	7.2	5.4	5.9
	10	7.8	8.2	6.8	5.4	5.9
	20	7.5	6.3	5.9	5.0	5.4
Frankfort	5	---	6.0	7.7	4.7	5.6
	10	---	6.0	7.6	4.6	5.4
	20	---	6.3	7.2	4.5	5.1
West Lafayette	4	---	---	6.2	---	---
	20	---	---	5.5	---	---
	50	---	---	3.2	---	---

* pH of 0.01 M $Ca(NO_3)_2$ + Cu solution after equilibration with sludge previously extracted with reagents shown.

Table 13.19. The pH values of 0.01 M $Ca(NO_3)_2$ - nickel solutions after equilibration with Anderson, Frankfort and West Lafayette sludges which had been extracted with reagents used to remove specific components.

Type of sludge	Nickel added	Extractants used to remove specific sludge components				
		NH_4F	$Na_4P_2O_7$	Na Citrate - $NaHCO_3$ - $Na_2S_2O_4$	NaOAc	H_2O_2
	mg/g			pH*		
Anderson	5	8.1	7.2	7.0	5.5	6.3
	10	7.8	7.3	7.3	5.4	6.7
	20	7.6	7.1	7.2	5.2	6.8
Frankfort	5	---	6.3	7.5	4.8	6.2
	10	---	7.1	7.5	4.8	6.4
	20	---	7.1	7.3	4.7	6.2
West Lafayette	4	---	---	6.8	---	---
	20	---	---	6.3	---	---
	50	---	---	5.7	---	---

* pH of 0.01 M $Ca(NO_3)_2$ + Ni solution after equilibration with sludge previously extracted with reagents shown.

Table 13.20. The pH values of 0.01 M $Ca(NO_3)_2$ - zinc solutions after equilibration with Anderson, Frankfort and West Lafayette sludges which had been extracted with reagents used to remove specific components.

Type of sludge	Zinc added	Extractants used to remove specific sludge components				
		NH_4F	$Na_4P_2O_7$	Na Citrate - $NaHCO_3$ - $Na_2S_2O_4$	NaOAc	H_2O_2
	mg/g	pH*				
Anderson	5	8.1	8.5	7.4	5.3	6.3
	10	7.9	6.7	7.1	5.3	6.0
	20	7.2	6.7	6.9	5.1	5.8
Frankfort	5	---	6.5	7.3	4.7	6.0
	10	---	6.4	7.7	4.7	5.9
	20	---	6.3	7.2	4.5	5.6
West Lafayette	4	---	---	6.9	---	---
	20	---	---	6.1	---	---
	50	---	---	5.2	---	---

* pH of 0.01 M $Ca(NO_3)_2$ + Zn solution after equilibration with sludge previously extracted with the reagents shown.

Table 13.21. Comparison of metal retention in three sludges before and after extraction with reagents designed to remove specific components (metals added at rate of 10 mg/g sludge).

Metal added	Type of sludge	Residue from extraction with:					
		Nothing	H_2O_2	CDB	NaOAc	$Na_4P_2O_7$	NH_4F
		------------ % of added metal retained ------------					
Cd	Anderson	85	18	58	45	60	99
	Frankfort	90	2	24	0	98	--
	West Lafayette	83	--	40	--	--	--
Cu	Anderson	89	11	32	42	64	99
	Frankfort	96	21	4	32	78	--
	West Lafayette	86	--	13	--	--	--
Ni	Anderson	61	0	8	9	89	70
	Frankfort	59	7	0	23	100	--
	West Lafayette	52	--	0	--	--	--
Zn	Anderson	74	0	18	31	99	89
	Frankfort	77	19	21	30	98	--
	West Lafayete	77	--	73	--	--	--

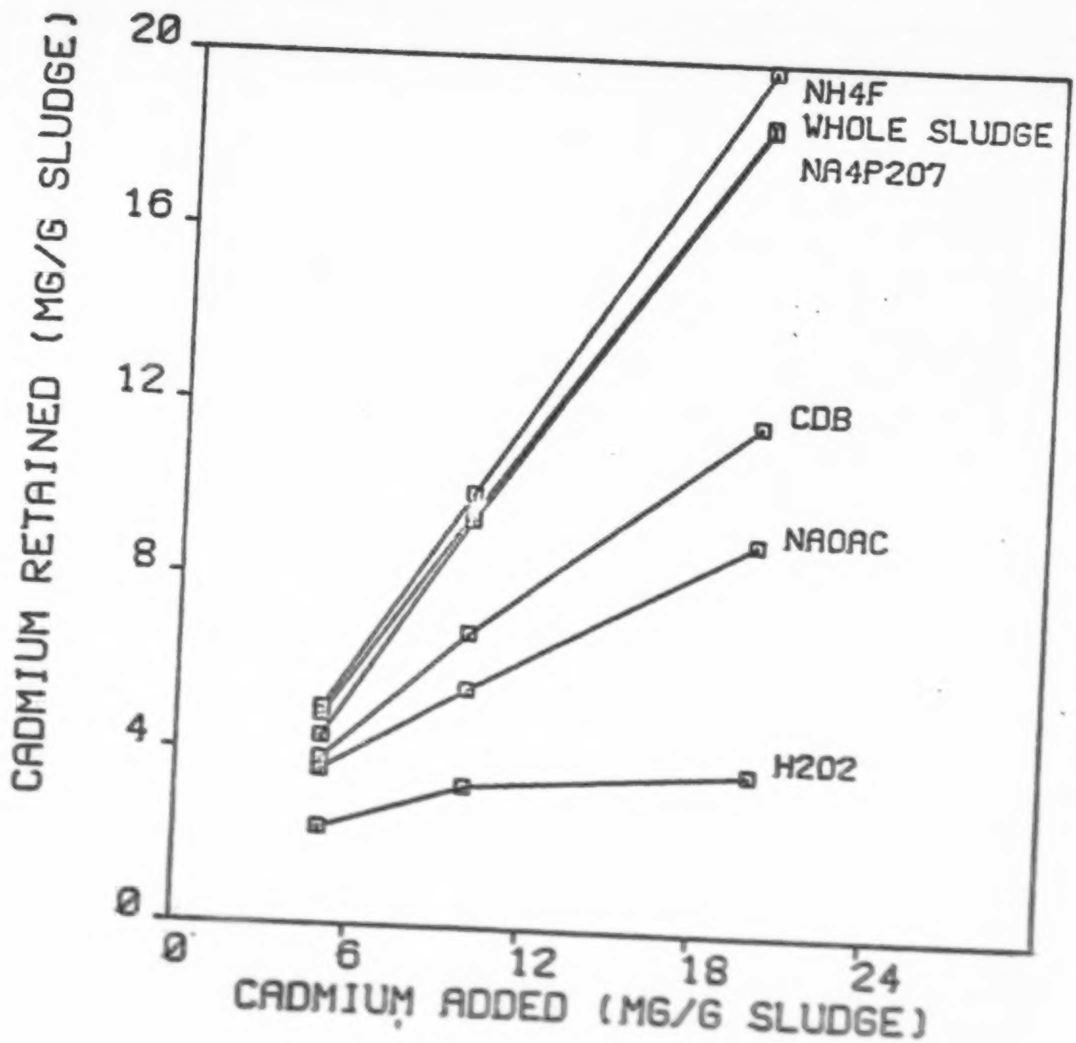

Figure 13.1. Cadmium retention by Anderson sludge before and after selective removal of components.

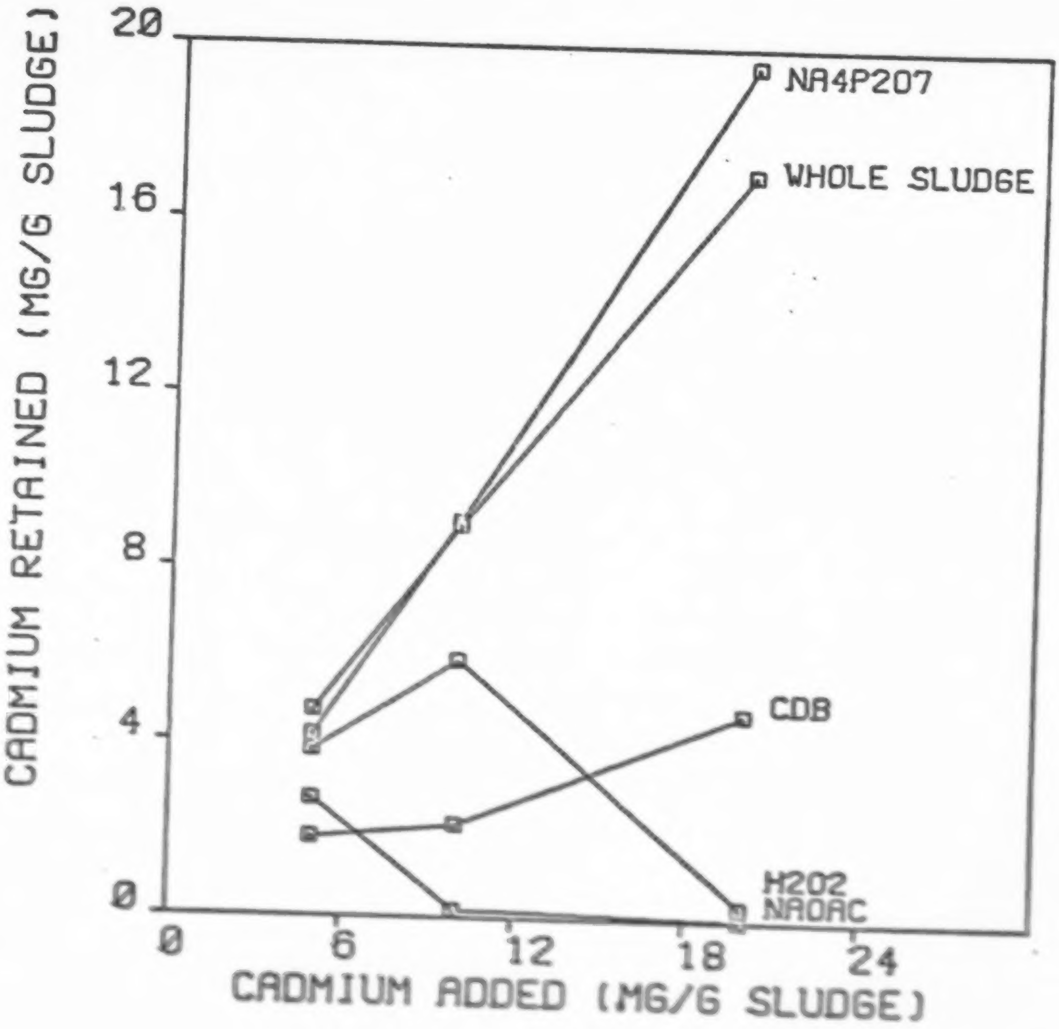

Figure 13.2. Cadmium retention by Frankfort sludge before and after selective removal of components.

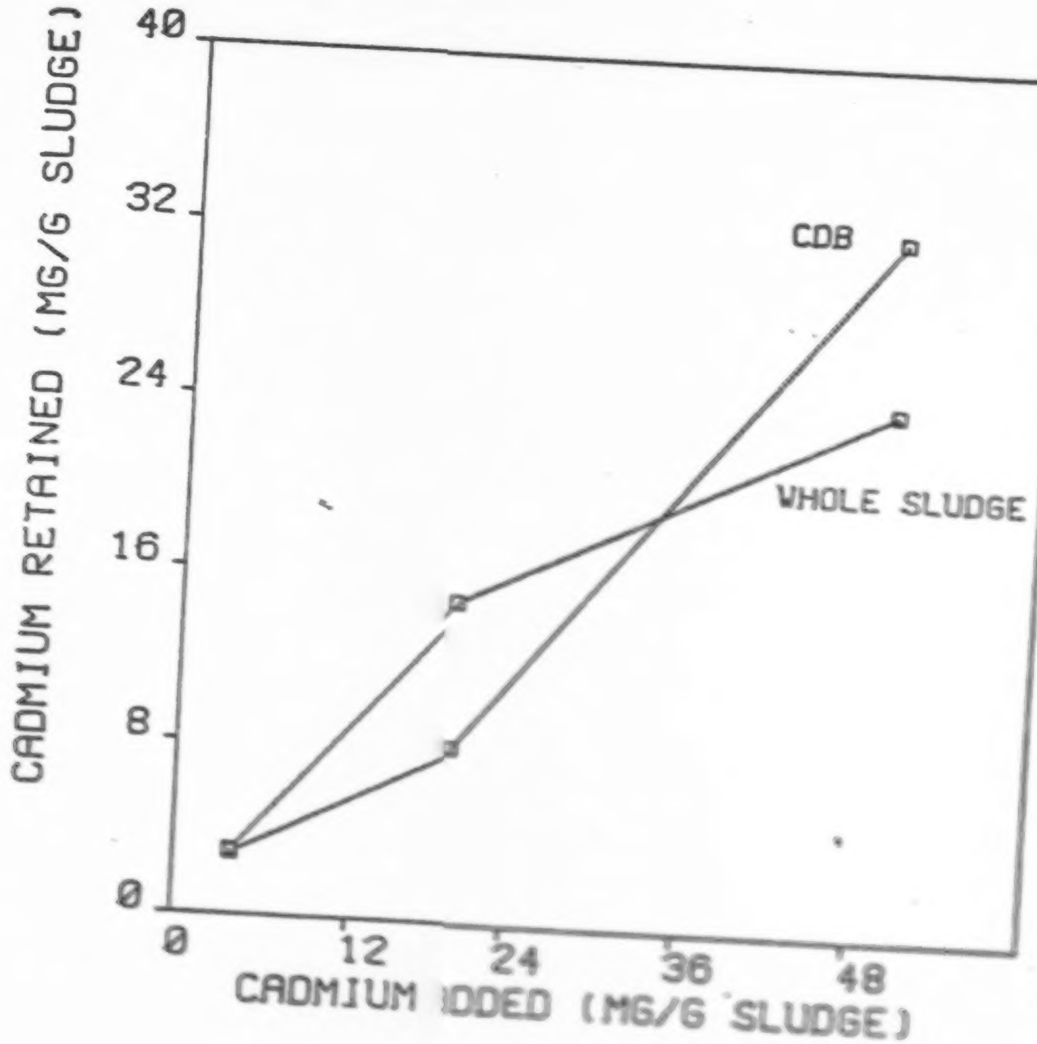

Figure 13.3. Cadmium retention byWest Lafayette sludge before and after CDB extraction.

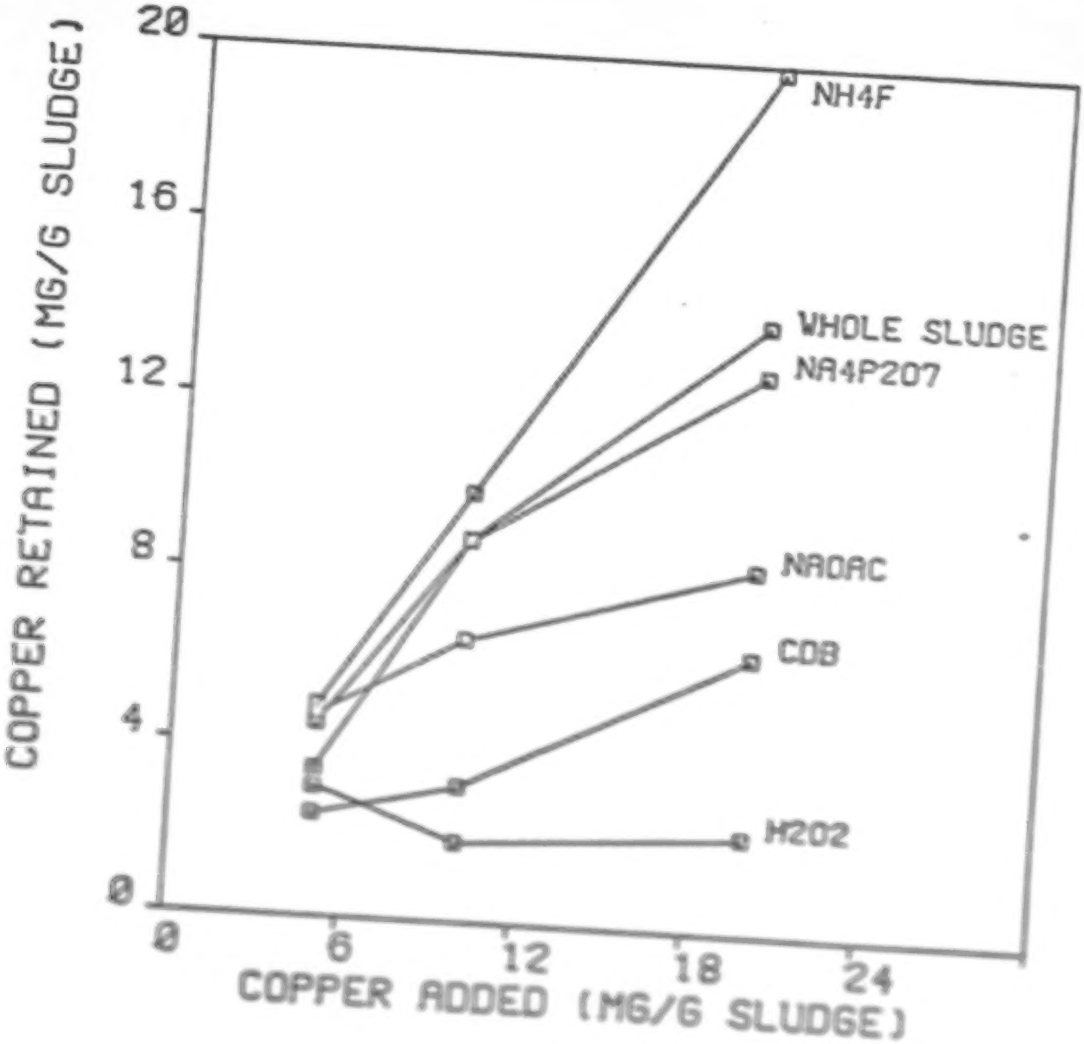

Figure 13.4. Copper retention by Anderson sludge before and after selective removal of components.

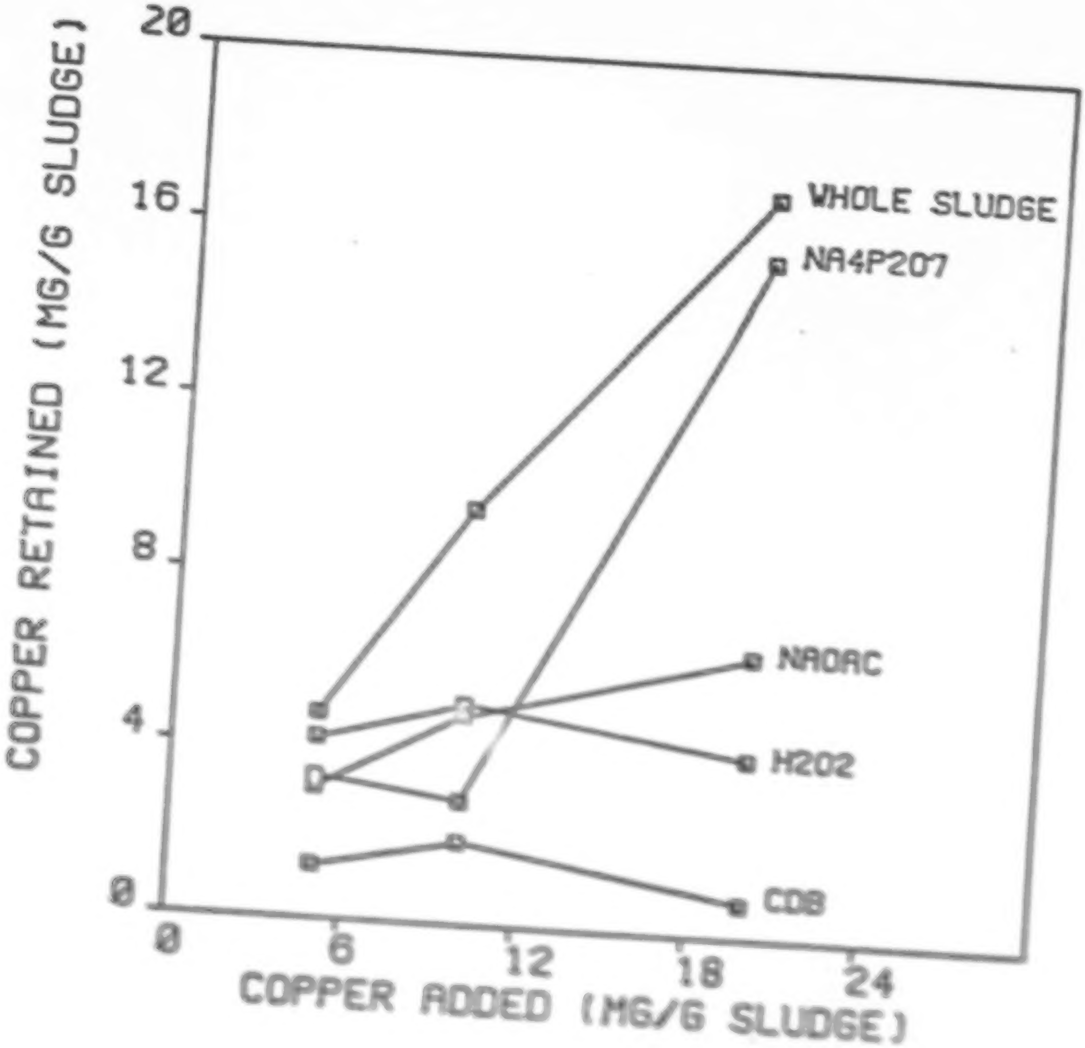

Figure 13.5. Copper retention by Frankfort sludge before and after selective removal of components.

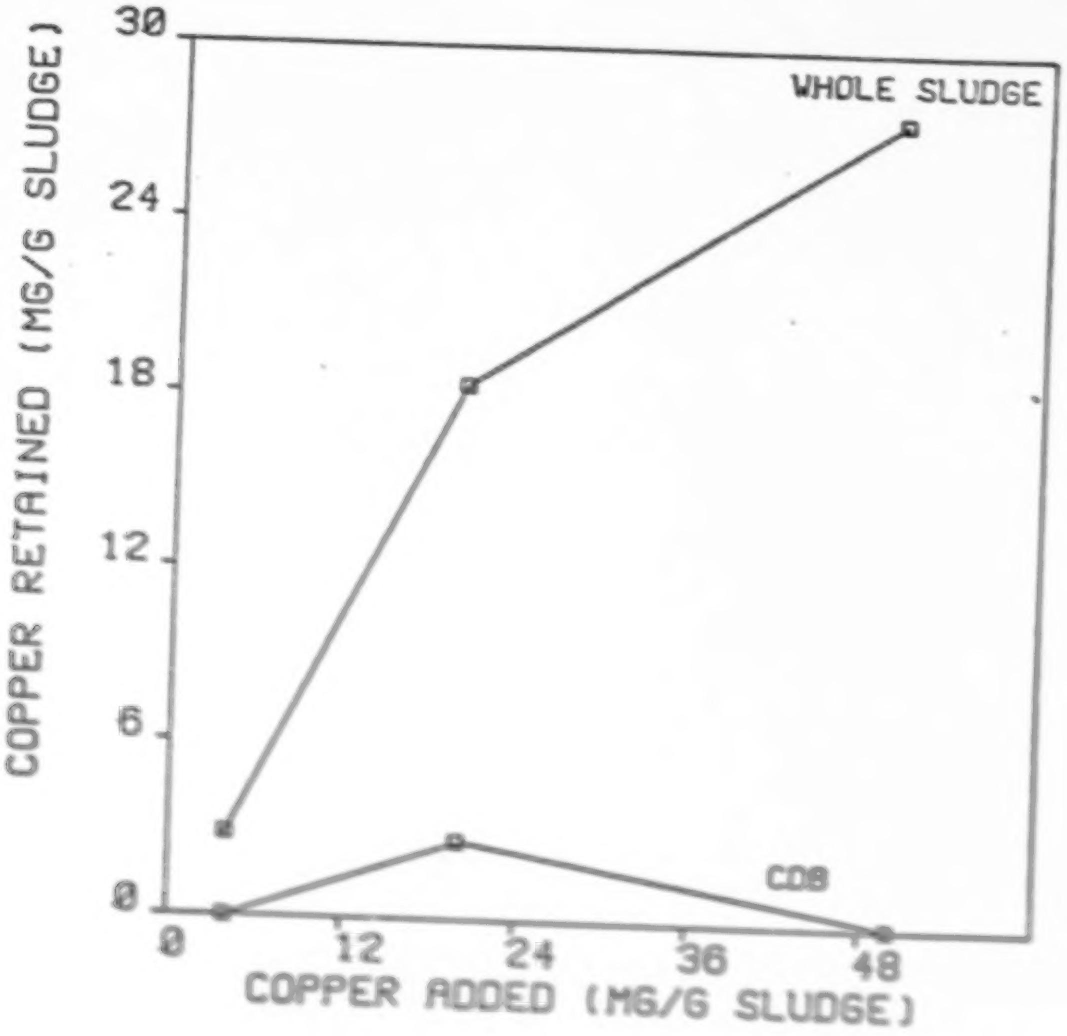

Figure 13.6. Copper retention by West Lafayette sludge before and after CDB extraction.

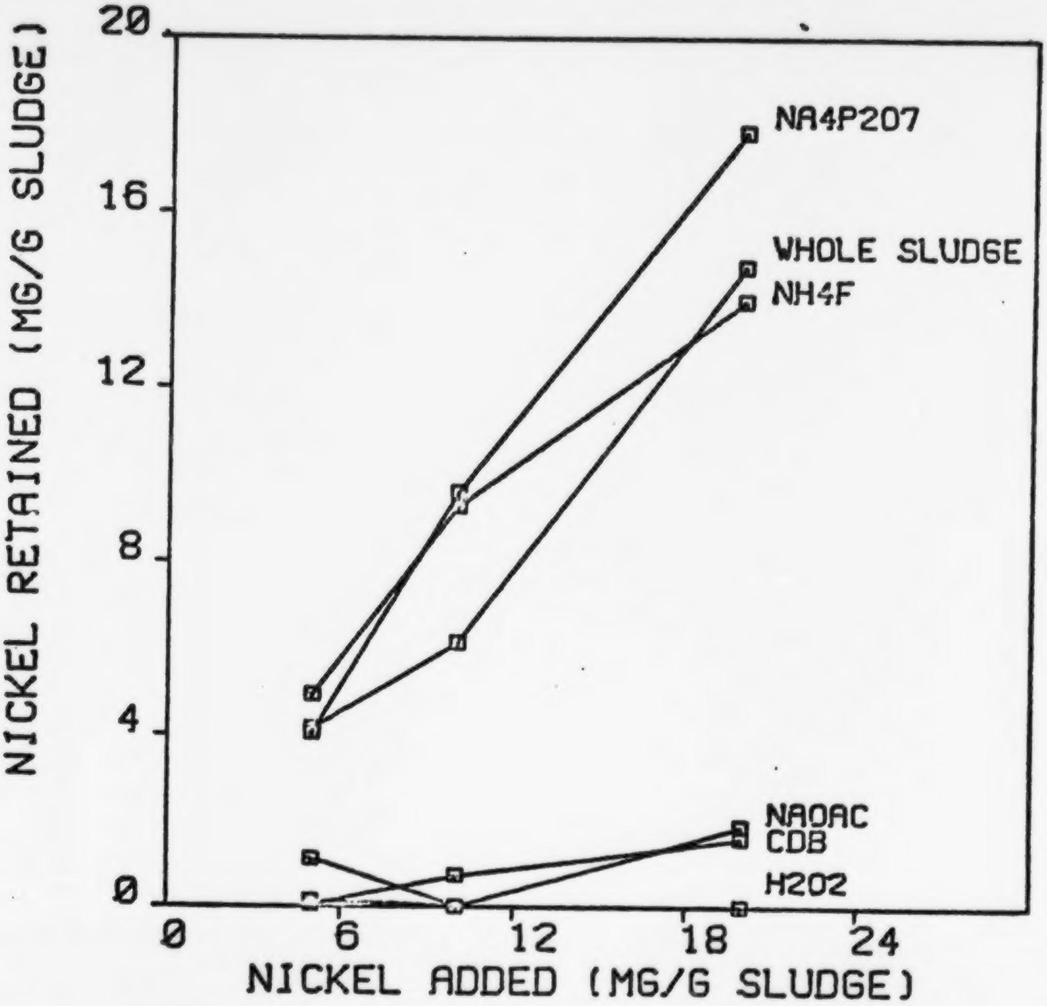

Figure 13.7. Nickel retention by Anderson sludge before and after selective removal of components.

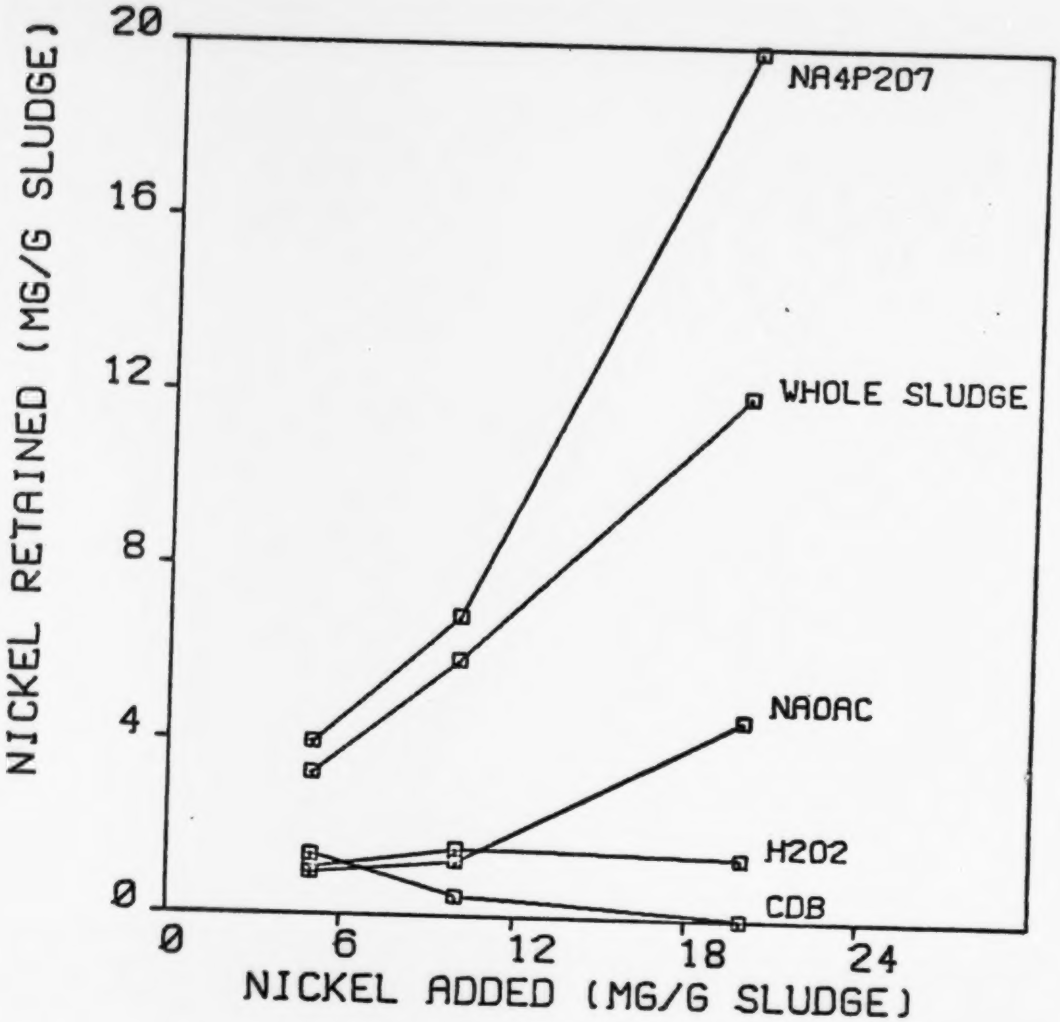

Figure 13.8. Nickel retention by Frankfort sludge before and after selective removal of sludge components.

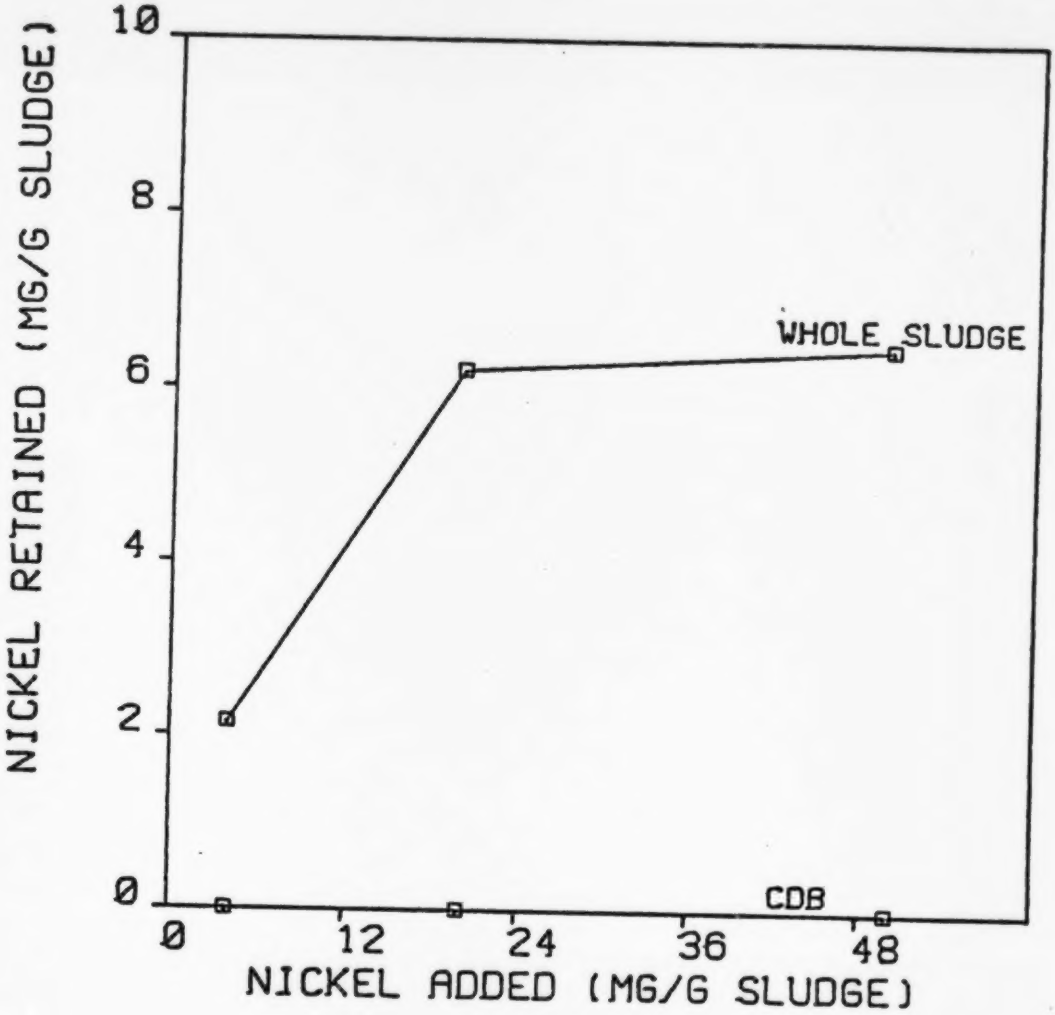

Figure 13.9. Nickel retention by West Lafayette sludge before and after CDB extraction.

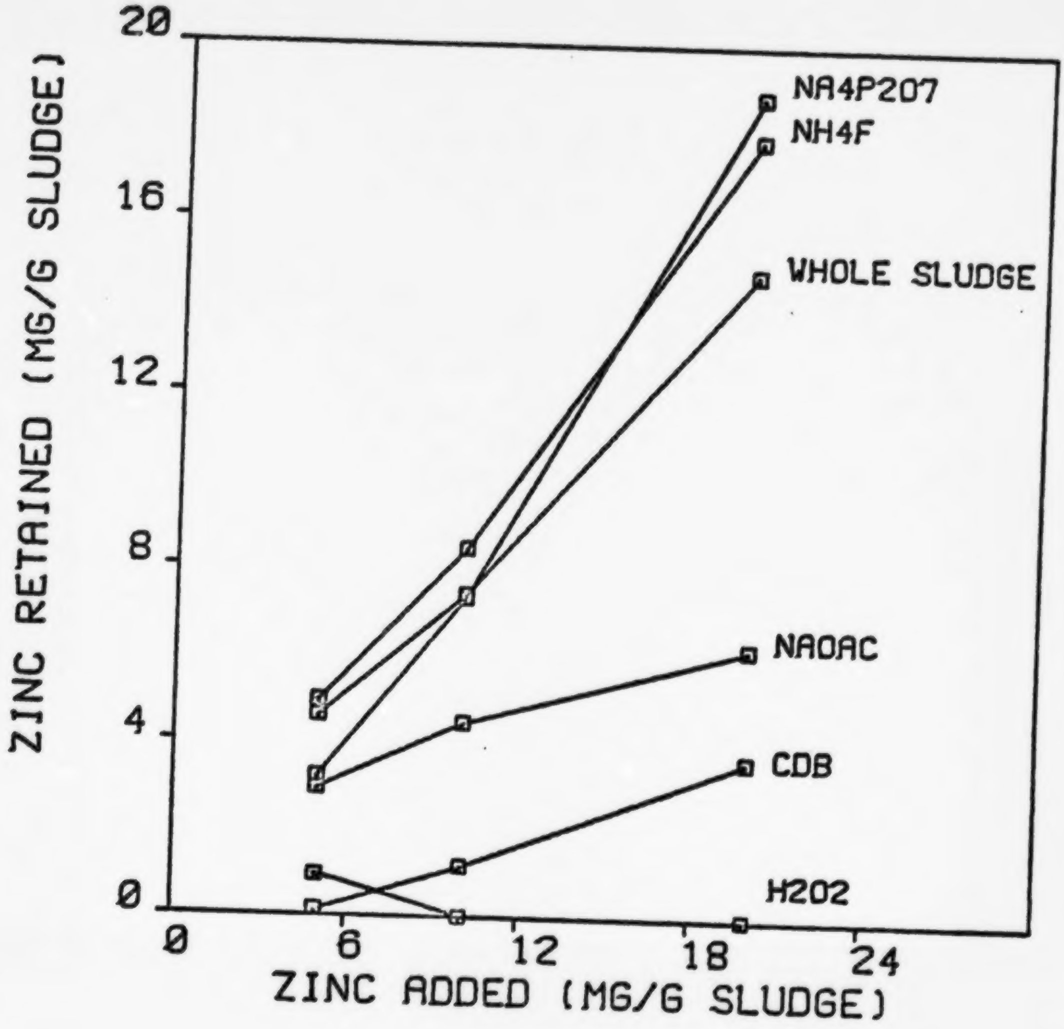

Figure 13.10. Zinc retention by Anderson sludge before and after selective removal of components.

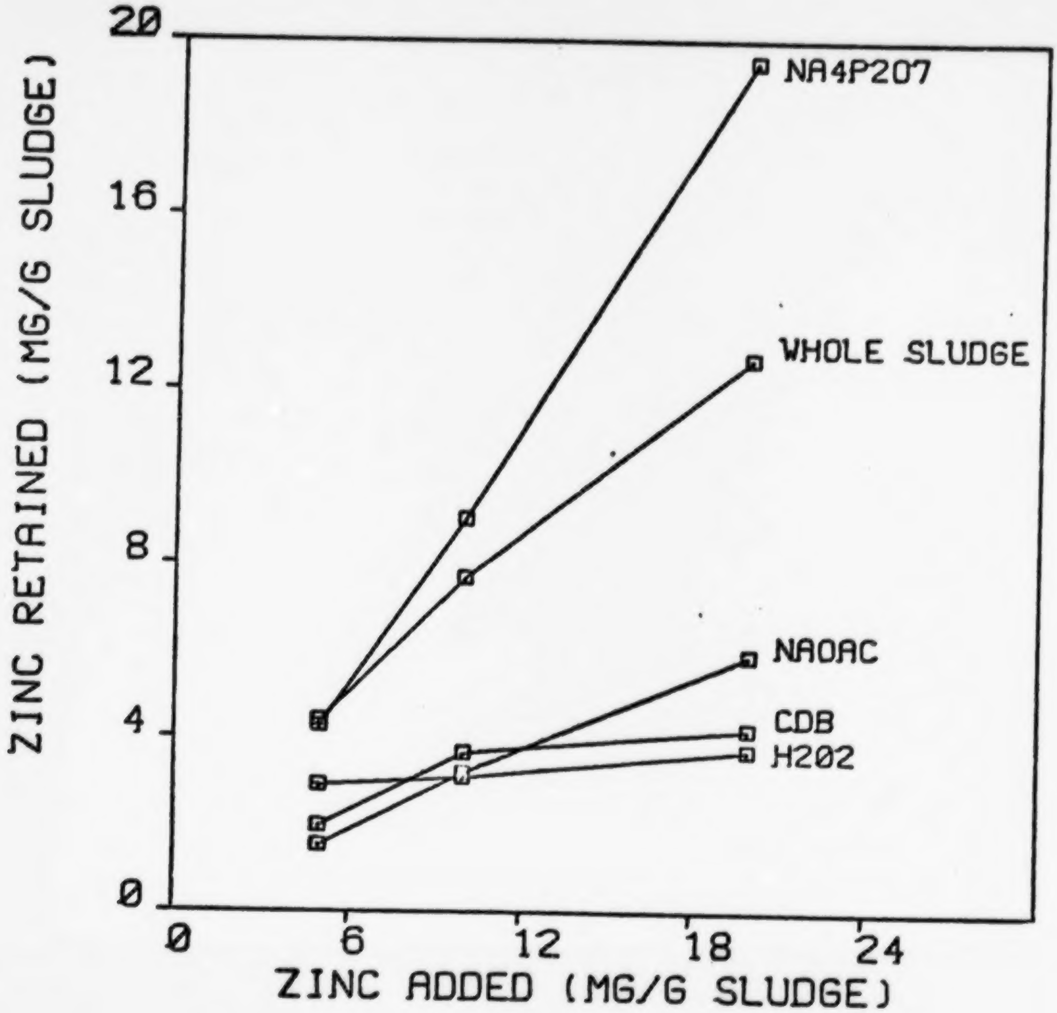

Figure 13.11. Zinc retention by Frankfort sludge before and after selective removal of sludge components.

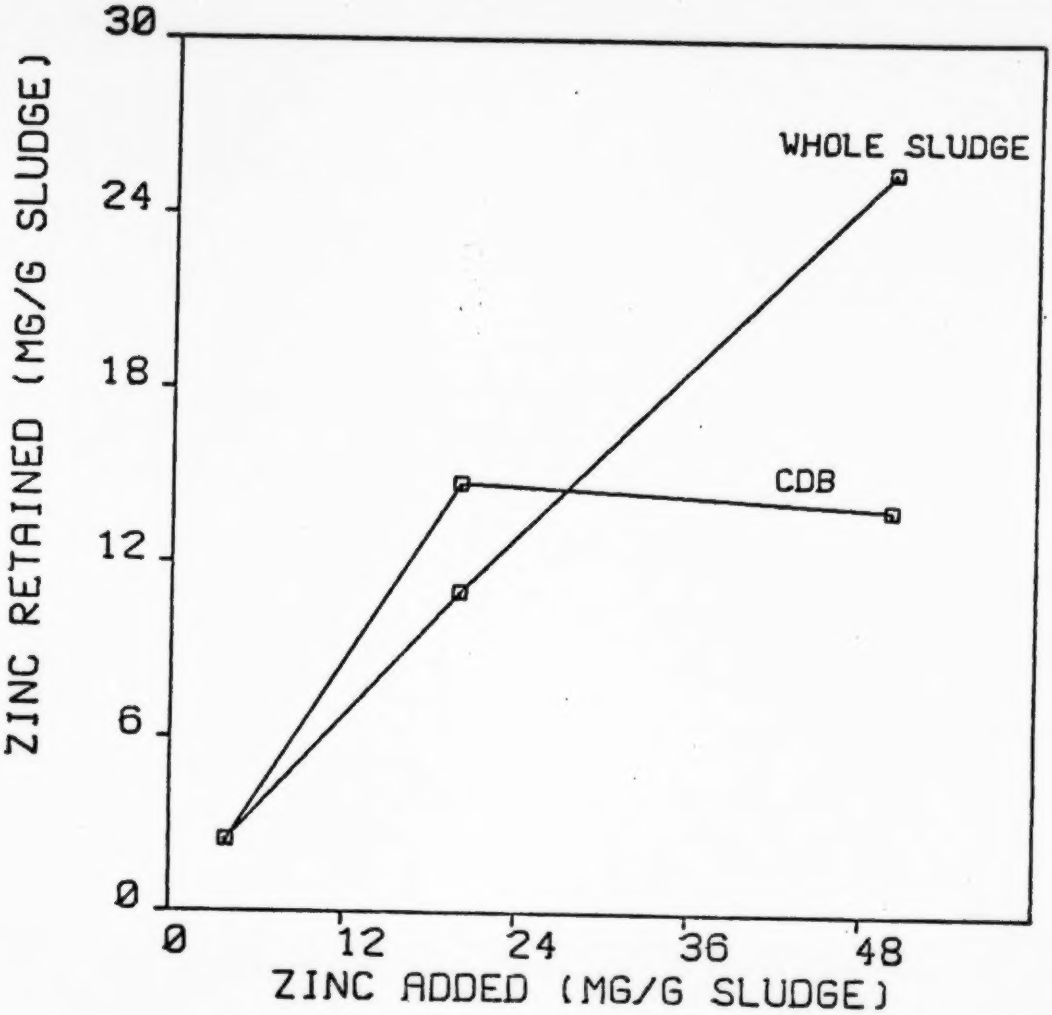

Figure 13.12. Zinc retention by West Lafayette sludge before and after CDB extraction.

SECTION 14

EFFECT OF SEWAGE SLUDGE ON THE COMPOSITION OF MILLING FRACTIONS OF OAT, CORN, WHEAT AND SOYBEAN GRAIN

INTRODUCTION

One of the major concerns arising from applications of sewage sludges on agricultural soils is the potential for increased concentrations of potentially toxic metals in crops and subsequently in the human diet. Sewage sludges contain not only macro- and micronutrients required for plant growth, but also toxic metals such as lead (Pb), nickel (Ni) and cadmium (Cd) (Page, 1974; Sommers, 1977). The concentration of metals in crops grown on soils treated with sewage sludge is a complex function of sludge, soil, plant and environmental factors. This topic has been the subject of several recent reviews (Chaney and Giordano, 1977; CAST, 1976; CAST, 1980; Baker et al., 1977; Cataldo and Wildung, 1978).

Of the metals applied to soils in sewage sludge, Cd has received the greatest attention due to its uptake by crops and potential for toxicity to humans and animals (CAST, 1980). This concern has resulted in the U.S. Environmental Protection Agency establishing regulations on the amounts of Cd that can be applied to soils used for crop yields. It is also desirable to limit the total amounts of zinc (Zn), Pb, Cu and Ni applied to soils (Knezek and Miller, 1976).

The majority of research evaluating the effects of sewage sludge applications on metal concentrations in crops has involved analysis of whole grains, fruits or tubers. In many cases, grains are processed before they enter human foods. To evaluate the influence of sludge application on metals entering the human diet, the distribution of metals in the various fractions of processed grains must be known in addition to the concentration in the whole grain. Dry-milling of corn grain has indicated that 49% of Zn and Cd, 36% of Cu, 44% of Ni, 55% of Fe and 57% of Mn was found in the endosperm (Hinesly et al., 1979). Similar analysis of wheat grain revealed that the flour contained from 14% of the Zn to 55% of the Pb. These authors suggested that sewage sludge additions to soils would significantly increase metal concentrations in fractions of corn and wheat used in animal rations, while only nominal increases in metal levels would result in the fractions of these grains consumed directly by man.

The objective of this study was to evaluate the distribution of metals in oat, corn, wheat and soybean grain grown on soil treated with municipal sewage sludges.

MATERIALS AND METHODS

Experimental Design

Field plots were established in the fall of 1976 at the Purdue University Agronomy Farm to evaluate the effect of applying excessive amount of heavy metals contained in sewage sludge on the yield and composition of several agronomic crops. Sewage sludges selected for study were obtained from sewage treatment plants in Anderson, Marion and Frankfort, Indiana. Since these sludges contained elevated metal levels, significant increases in soil metal concentrations were obtained with sludge application rates of $\leq$ 448 metric tons/ha. All sludges were subjected to anaerobic digestion and then dewatered to approximately 50% solids on sand-drying beds or equivalent. The composition of the sewage sludges (dry weight basis) used are shown in Table 14.1. The sludges were applied to the surface of 4.6 x 12.2 m plots (4 replicates) and incorporated into the soil by plowing to a depth of 20 cm. The soil was a Chalmers silty clay loam with the following properties: organic C, 2.3%; total N, 0.18%; cation exchange capacity, 23 meq/100 g; pH, 5.6. The crops grown were: 1977-oats (*Avena sativa* L. var. 'Noble'); 1978-corn (*Zea mays* L. var. 'Becks 65x'); 1979-winter wheat (*Triticum aestivum* var. Beau); 1979-soybeans (*Glycine max* L. var. 'Amosoy 71'). Supplemental N fertilizer was applied to the corn and winter wheat plots while no N was applied to oats and soybeans.

All grain samples were collected from each replicate plot, dried at 60°C for 48 hours and stored prior to analysis.

Test Weight

The weight, per Winchester bushel to the nearest 0.1 pound, was determined by Method 84-10 of the AACC Approved Methods (1976). The pounds per bushel test weight was converted to kilograms per hectoliter by multiplying by a factor of 1.29.

Processing Procedures

Samples of oat, corn, wheat and soybean grain were processed by typical commercial procedures using laboratory equipment. A subsample of grain from each replicate plot was subjected to the processing procedures.

Analytical Procedures

Protein content was determined by the Kjeldahl method as modified by Noel (1979). Ash, fat and moisture contents were determined as described in Methods 08-01, 30-25 and 44-40, respectively, of the AACC Approved Methods (1976).

For metal analysis, samples (2.00 ± 0.01g) of ground grain (< 40 mesh) or grain fractions were placed in 25 x 200 mm pyrex tubes, treated with 10 ml of concentrated HNO_3 and maintained at room temperature for 16 to 20 hrs. The samples were then placed in an aluminum block, initially at room temperature, and then gradually heated to 130°C. Heating at 130°C was continued until about 0.25 ml of HNO_3 remained. After cooling the samples, 3 ml of a 60% $HClO_4$:conc. H_2SO_4 (12:1, v/v) mixture were added and boiled gently in an aluminum block preheated to 210°C until evaporation of the $HClO_4$ was complete. After cooling, the samples were diluted to 10 ml with deionized water. The digests were analyzed for Cd, Zn, Ni, Cu, Fe and Mn with a Varian AA-6 flame atomic absorption spectrophotometer fitted with a background corrector (used for Cd and Ni).

Appropriate controls and standards were prepared. All reagents used were analytical grade and solutions were prepared with distilled deionized water. Glassware was initially washed in 10% HNO_3 before use. All data presented are the average of triplicate determinations on grain samples obtained from four replicate field plots.

RESULTS AND DISCUSSION

Oats

The effect of sewage sludge application on the test weight and protein and ash contents of whole oats, groats and hulls is shown in Table 14.2. The test weight was not significantly altered by sludge additions (range of 45.6 to 48.2 kg/ha) except for the Anderson sludge applied at 224 and 448 metric tons/ha. As expected, essentially all protein was contained in the groat fraction. The protein content of both whole oats and groats increased with increasing amounts of sludge applied. This result is consistent with increased levels of plant available N in the soil amended with sludge. The ash content of oats and oat fractions tended to decrease with increasing amounts of sludge applied. The two above parameters are general characteristics of oats that are useful in evaluating their quality as a feed stuff. As such, sludge applications have resulted in beneficial changes, e.g., increased protein content. Protein levels were significantly enhanced by all application rates of the Frankfort sludge, the 168 metric ton/ha of the Marion sludge, and the 224 and 448 metric ton/ha of the Anderson sludge. Since sludges contain heavy metals that are potentially detrimental to man or animals, the metal content of oats grown on sludge amended soils must be known to adequately evaluate quality in addition to the routinely determined parameters discussed above.

The metal concentrations of whole oats, groats and hulls are presented in Tables 14.3 to 14.5. Also shown is the rate of sludge application along with the amount of each metal applied. When the rates of metals applied are compared with current recommendations for Zn, Cu and Ni (U.S. EPA, 1977) and regulations for Cd (U.S. EPA, 1979), it can be seen that the amounts of sludge applied are often far in excess of those recommended or allowed. The experimental design was adopted to evaluate the validity of current limits on metal additions to soils through a single application of different sludges, rather than to determine optimum sludge application rates from an agronomic standpoint. As a result, the effects of sludge addition on the metal content of oats are not representative of those occurring when sludge is applied to soils at rates recommended for agronomic crops.

Sludge applications increased the levels of Cu, Ni, Zn, Cd and Fe (Tables 14.3 to 14.5) in whole oats while Mn concentrations were decreased

(Table 14.5). Numerous studies have shown that increases in metal concentrations of various crops are related to the amount of metal applied in sludge and that soil properties, such as pH, strongly influence metal uptake by plants (e.g., CAST, 1976; CAST, 1980; Chaney and Giordano, 1977; Sommers, 1980). The sludges used in this study contained calcium carbonate resulting in an increase in soil pH from 5.6 to ~ 7.4. This pH change is undoubtedly responsible for the depressed Mn concentrations in oats grown on sludge treated plots. Even though the plant availability of Cu, Ni, Zn, Cd and Fe is also inversely related to soil pH, the amount of these metal applied to soils in a plant available form must have more than compensated for the decreased availability caused by the increase in pH.

The groats contained greater concentrations of Cu, Ni, Zn, Cd and Mn than the hulls. The Fe content of groats and hulls was approximately the same. The relative enrichment of metals in the groat fraction of oats was calculated from the data shown in Tables 14.3 to 14.5. The ratio of metal in groats:hulls in decreasing order were: Ni, 3.2; Cd, 2.3; Mn, 2.0; Cu, 1.5; Zn, 1.4; and Fe, 1.2. These values are the averages obtained from all treatments. Inspection of the data reveals that the ratio of metal content in groats:hulls tends to increase with increasing amounts of sludge-borne metals applied to the soil, but exceptions to this general trend can be readily found. Since all oat samples were comprised of approximately 75% groats and 25% hulls, the majority of metals assimilated by oats are contained in the groat fraction rather than the hulls. Thus, ash content is not a good indicator of trace metal content in oat fractions. As shown in Table 14.2, the ash content of hulls was approximately 2 to 3 times that of the groats whereas all trace metals were present in higher concentrations in the groats.

The concentrations of trace metals in whole oats, groats and hulls followed a similar pattern. In general, the concentrations decreased in the following order: Zn > Fe > Mn > Ni > Cu > Cd. This pattern was similar in oats grown on non-amended or sludge-amended soils.

In summary, the processing of oats into groat and hull fractions revealed that sludge applications increased the trace metal concentrations of both fractions. The total intake of trace metals from oats used in the diet will be similar if whole oats or only the groat fraction is consumed. However, it must be realized that total metal content may not be directly and linearly related to bioavailability of a metal (Erdman, 1980). Although sludge applications altered the distribution of trace metals in groats and hulls, the changes were relatively minor. To estimate the effect of sludge applications on the metal content of foods prepared from oats, analysis of whole oats will provide a satisfactory indicator of the metal content in all products using only groats. These results are different from those of Hinesly et al. (1978) where the trace metal content of corn and wheat endosperm fractions contained lower metal levels than the bran or animal feed fractions.

Corn

The yields of corn grain were not adversely affected by sludge application rates ranging from 56 to 448 metric tons/ha (data not presented). The application of fertilizer N on all sludge-treated plots insured that a N deficiency did not cause elevated metal levels in corn grain through decreased dry matter accumulation. Corn grain yields were increased to a limited extent by fertilizer treatments. Considering all fertilizer and sludge-treatments, the average yield of corn grain was 8.07 metric tons/ha with a standard deviation of 0.89 metric tons/ha.

No processing problems (tempering, degermining and roller milling) were encountered with the corn grown on either fertilizer or sludge-treated plots. The yields of corn dry-milled products derived from corn grown on both sludge and fertilizer-treated plots were similar. The mean percentages of the whole corn obtained in the various fractions were as follows: grits, 31; meal, 18; flour, 21; germ, 11 and; hulls, 19. Overall these product yields are consistent with those expected for dry-milled yellow dent corn (Young, 1981).

The protein, ash and fat content of whole corn and the dry-milled fractions are shown in Table 14.6. The protein content of whole corn was increased slightly by both fertilizer N and sewage sludge applications. The maximum protein content was obtained with the largest sludge application rate used (448 metric ton/ha of sludge 1).

The protein content of all corn fractions was also increased in a similar manner by application of sewage sludge. As expected, the concentration of protein in the germ was approximately double that found in whole corn. The protein content of the corn fractions decreased in the following order: germ >> hulls > grits = meal > flour. By weight the grits fractions accounted for 31% of the whole corn and contained from 25 to 30% of the total protein. While the germ and hull fractions comprised only 11% and 19% of the whole grain weight, respectively, each contained > 20% of the protein present in whole corn. The concentrations of ash and fat in the fractions generally followed the above trend but the concentrations in the germ were considerably higher than in the other fractions. However, within each fraction, ash and fat content were not significantly affected by sludge and fertilizer applications. The germ fraction typically contained > 50% of the total fat and ash present in whole grain. This data indicates that sludge application to this soil did not cause major changes both in the concentration or the distribution of protein, fat and ash in whole corn or the corn products obtained by dry-milling.

The concentrations of Cd, Ni and Zn (Table 14.7) and Cu, Fe and Mn (Table 14.8) were not significantly increased in corn grain by application of sewage sludge to soil, even though the sludges contained appreciable amounts of these metals. Two factors are likely responsible for the minor changes observed in metal concentrations of corn grain. It has been shown in numerous studies that corn grain obtained from sludge-treated soils does not show markedly elevated metal concentrations (Chaney and Giordano, 1977; CAST, 1976, 1980; Sommers, 1980). Secondly, the three sludges used in this study contained free $CaCO_3$ which resulted in the soil pH increasing from 5.6 to $\geq$ 7.0. Even though sludge applications increased the concentrations of Zn, Cd, Cu and Ni in the soil, the resultant rise in soil pH undoubtedly minimized metal uptake by corn. In addition, the pH increase may have also decreased the plant availability of indigenous Zn, Cu, Fe and Mn in the soil.

The Cd concentration in whole corn grown on either fertilizer or sludge-treated soil was < 0.05 mg/kg, (Table 14.7). It should be noted all Cd additions are far in excess of the maximum allowable total Cd additions for this soil (20 kg Cd/ha; U.S. EPA, 1979). Therefore, the current regulations afford adequate protection against the accumulation of significant Cd levels in food products derived from corn grain. These results are consistent with other studies showing minimal translocation of sludge-borne Cd into corn grain (CAST, 1976, 1980).

Detectable concentrations of Ni were found in most samples of whole corn, and in germ and hull fractions (Table 14.8). The increase in Ni levels in the hull fraction from corn grown on plots treated with sludge 1 tended to parallel the sludge application rate. In general, the germ fraction contained the highest concentrations of Ni with values ranging from < 0.20 to 4.93 mg/kg. It was not possible to calculate percent distributions for Ni because many fractions contained concentrations below the detection limit of the procedure used.

Sludge applications to the soil did not markedly increase Zn levels in whole grain (range of 20.2 to 28.6 mg/kg) (Table 14.7). The concentrations of Zn in the germ ranged from 3 to 8 times those found in the whole grain. Zinc in the germ was increased from 66 to 113 mg/kg by fertilizer additions to the soil, whereas increasing application rates of (56 to 448 metric tons/ha) sludge 1 decreased Zn content in germ from 193 to 68 mg/kg. The whole grain and hull fraction possessed similar concentrations of Zn while the grits, meal and flour fractions were depleted of Zn. Of the total Zn in whole grain, approxiamtely 52 and 20% were present in the germ and hull fractions, respectively.

The Cu, Fe, and Mn concentrations in whole grain and corn fractions were similar for all sludge and fertilizer treatments (Table 14.8). The levels in whole grain showed the following ranges: Cu, 1.82 to 2.32 mg/kg; Fe, 14.7 to 20.6 mg/kg and; Mn, 2.20 to 3.78 mg/kg. As shown previously for Ni and Zn, the majority of Cu, Fe and Mn accumulated by corn grain were found in the germ fraction. The concentration ranges found in the germ fraction were: Cu, 5.41 to 21.00 mg/kg; Fe, 35.6 to 79.3 mg/kg and; Mn, 2.03 to 15.50 mg/kg. The hull fraction was the second most abundant fraction for these metals. The fertilizer treatments resulted in the greatest concentrations of Fe and Mn in the germ fraction, likely because the soil pH was lower than that found after sludge application.

In summary, the concentrations of protein, ash, fat, Zn, Ni, Cu, Fe and Mn in corn grain and fractions obtained by dry-milling were unaffected by applications of sewage sludge to soils. The relative concentration and percent distribution of metals in corn grain fractions generally decreased in the following order:

germ > hulls > flour > meal = grits.

This pattern was exhibited by Zn, Ni, Cu, Fe and Mn concentrations. The overall percentage of the total Zn, Cu, Fe and Mn present in the germ fraction was 65, 49, 30 and 25, respectively. The hull fraction contained

20, 25 21 and 20% of the total Zn, Cu, Fe and Mn, respectively. Thus, greater than 45% of the trace metals in the corn grain were found in dry-milled by-products (hull and germ fractions) that do not directly enter the human food chain. This suggests that, even if use of sewage sludge on cropland results in elevated concentrations of some potentially toxic metals (e.g., Cd, Ni) in corn grain, the metal content of human food products will be impacted to a much lesser extent than those consumed by animals. Unfortunately, essential trace metals such as Cu, Zn, Fe and Mn are also present in lower concentrations in the grits, flour and meal than in the germ and hull fractions.

Wheat

The application of increasing amounts of sewage sludge to soil resulted in decreasing yields of wheat grain (Table 14.9). The only exception to this trend was the 56 metric ton/ha application of sludge 1 which caused a slight, but not statistically significant, increase in yield. In comparison to the untreated soil, a statistically significant yield decrease was caused by an application rate of 224, 168 and 112 metric tons/ha for sludge 1, 2 and 3, respectively. These sludge application rates correspond to a yield decrement of 26 to 43%. Similarly, significant yield reductions occurred at a 18 to 26% decrement for wheat grown in soils treated with metal salts plus sewage sludge (Mitchell *et al*., 1978). In view of the large amounts of metals added to the soil, it is likely that wheat yields were reduced through either a direct metal toxicity or a metal-induced Fe or Mn deficiency (see discussions by chaney and Giordano, 1977; Chaney, 1980; Mitchell *et al*., 1978). Even though sludge applications reduced wheat yields, there was minimal affects on the test weight, thousand kernel count and particle size index (Table 14.9).

The protein content of wheat grain was increased by all rates of sewage sludge application (Table 14.9). For all three sludges, the protein content was directly related to the application rate and, as discussed above, inversely related to grain yield. Significant increases in protein were found at application rates of 112, 112 and 56 metric tons/ha for sludge 1, 2, and 3, respectively. As expected, the protein content of bran was greater than that of whole wheat grain and responded to sludge applications in a similar manner as wheat grain (data not presented). Continued N uptake along with decreased dry matter accumulation would explain the increased protein content of wheat following sludge applications. Adequate N availability was insured by applying urea in early spring to supplement the inorganic N released by decomposition of organic N in sewage sludges. The ash content of wheat ranged from 1.90 to 2.12% while brain contained from 4.32 to 4.85% (data not presented). For the majority of treatments, there was a similar ash content in wheat grown on untreated and sludge-treated soils.

The effect of sludge application on the Zn, Cd, Cu, Ni, Fe and Mn content of wheat, bran and flour is shown in Table 14.10. Application of sewage sludge resulted in significant increases of Zn, Cd, Ni and Mn in wheat grain. In general, the increase in metal concentrations paralleled the amount of metal applied to the soil. Because the sludges contained variable amounts of calcium carbonate that increased soil pH (e.g., from 5.8

to $\geq$ 7.0) and because metal availability to plants is reduced at near neutral pH levels (CAST, 1976), a direct correspondence was not found between the metal levels in wheat and the sludge-borne metals applied to soil. The increase in soil pH may explain the minimal effect of sludge applications on Fe concentrations in wheat (Table 14.10). There was also a tendency for Cu concentrations in grain to be somewhat reduced for most sludge treatments. The concentrations ranges found in wheat grain were: Zn, 37.3 to 71.4 mg/kg; Cd, 0.47 to 4.43 mg/kg; Cu, 3.8 to 5.6 mg/kg; Ni, < 0.10 to 4.47 mg/kg; Fe, 29.8 to 43.2 mg/kg and Mn, 3.96 to 14.3 mg/kg.

The metal concentrations in the bran always exceeded those found in the flour (Table 14.10), typically by a factor of 5 to 10. In both bran and flour, there was a general tendency for metal concentrations to increase with increasing amounts of sludge applied. The results obtained are very similar to those of Hinesly et al. (1978) with respect to the effect of sludge applications on the metal content of grain, bran and flour and the distribution of metals in bran and flour. Metal concentrations in flour exhibited the following ranges: Zn, 7.5 to 12 mg/kg; Cd, 0.22 to 1.58 mg/kg; Cu, 0.96 to 2.64 mg/kg; Ni, < 0.10 to 0.63 mg/kg; Fe, 8.27 to 11.2 mg/kg and; Mn, 1.04 to 2.79 mg/kg. With the exception of Cd, the above metal concentrations are within the ranges presented for wheat flours by Czeiniejewski et al. (1964), Toepfer et al. (1972) and Waggle et al. (1967). For Cd, concentrations in the flour were not increased by application of sludge 2 even though from 14 to 42 kg Ca/ha were applied. In contrast, applications of sludge 1 (56 metric tons/ha = 16 kg Cd/ha) and 3 (56 metric tons/ha = 68 kg Cd/ha) resulted in significant increases in flour Cd, which was directly related to the amount of Cd applied. It should be noted that current regulations (U.S. EPA, 1979) would limit Cd additions to 20 kg/ha for the soil used in this study (i.e., approximately 60 metric tons/ha for sludge 1 and 2 and 25 metric tons/ha for sludge 3). In addition, the Cd content of wheat grown on untreated plots was 0.89 mg/kg whereas most studies report values of < 0.1 mg/kg. This difference may be related to the natural Cd level in the soil, an acid pH (5.8) and the cultivar of wheat grown.

The metal data presented in Table 14.10 along with flour yield (Table 14.9) can be used to assess the distribution of metals in bran and flour. Except for Ni where non-detectable levels limit such calculations, the percentage distributions in the flour 12%, 16%, 19%, 23% and 24% for Zn, Mn, Fe, Cd and Cu, respectively. In a similar study, Hinesly et al. (1979) reported values ranging from 14% for Zn to 43% for Mn. Sewage sludge applications did not cause a marked change in the relative proportion of metals found in the bran and flour. Thus, the effect of sludge applications on the metal content of flour used in the human diet can be approximated from a knowledge of the wheat grain metal content and a constant distribution function.

The effects of sewage sludge on the characteristics of flour and related baking parameters are presented in Table 14.11. The ash content of flour was not affected by sludge additions while significant increases in the protein content were observed. Protein content of flour was directly related to sludge application rate, increasing from 12.39% for the control to ~ 15.5% for the 168 to 448 metric tons/ha sludge treatments. It should be emphasized that all sludge treated plots also received a constant amount of urea. The increased plant available N is sludge treated plots was due to the mineralization of organic N. Alkaline water retention capacity measurements (data not presented) indicated that sludge applications had minimal effects on the pentosan content of the flour. Because of the increased protein levels, the expected decrease in mixograph mixing time and cookie diameter was observed for flour obtained from wheat grown on the sludge treatments.

In summary, the milling qualities of soft red winter wheat are not influenced to a significant extent by applying sewage sludge to soils. However, sludge applications increased flour protein levels and thus altered selected baking parameters. From 75 to 85% of the Zn, Mn, Fe, Cd and Cu was present in the bran rather than the flour, indicating that any adverse human health effects related to metals such as Cd can be minimized by utilizing food products prepared from flour as opposed to whole wheat.

Soybeans

Sewage sludge applications ranging from 56 to 448 metric tons/ha had minimal affects on soybean yield and test weight (data not presented). Soybean yields ranged from 2.93 to 3.32 metric tons/ha (average = 3.19 metric tons/ha). A significant yield reduction was found for the 168 metric ton/ha application of sludge 3 (2.75 metric tons soybeans/ha). For nearly all treatments, no significant impact was observed for the test weight of soybeans. As shown in Table 14.12, sludge applications also resulted in essentially no alteration of the protein, ash or fat content of the whole bean or hull and meal fractions. Thus, sludge application at metal loading rates considerably in excess of current guidelines did not alter the properties of soybean grain typically used as measures of grain quality. It should also be noted that sludge applications increased the levels of plant available N in soils and yet minimal changes in the protein content of both beans and meal were observed.

The concentrations of Zn, Cd, Cu, Ni, Fe and Mn in whole beans, hulls and meal are presented in Table 14.13. Sludge applications caused minimal changes in the concentrations of Zn, Cu, Fe and Mn in whole beans and fractions. However, both Cd and Ni levels were significantly increased by sludge additions. Cadmium concentrations were in excess of 3 mg/kg following application of sludge 3 while Ni values increased from 5 to 18 mg/kg. Metal concentrations in the hulls exceeded those in the meal for Zn, Cd, and Fe. The reverse trend was observed for Cu, Ni and Mn.

The approximate percentages of hulls, meal and oil was 6%, 76%, and 18%, respectively, and they were not significantly affected by sludge applications. Analyses of selected oil fractions indicated that a minimal amount of metals accumulated in the soybean were present in the oil. The percent distribution of fractions in conjunction with metal concentration data indicates that the majority of metals will be recovered in the meal. For most metals, analysis of the whole bean serves as a good index of the subsequent metal concentration found in the meal fraction. If elevated metal concentrations, especially Cd, in soybeans following sludge application are encountered, utilization of the meal as a component in animal rations will minimize any direct impact on human health. It should also be noted that essentially no changes in metal content of soybean grain were found when sludge applications rates were consistent with current recommended guidelines.

Table 14.1. Composition of sewage sludges*.

	Source of sludge		
	Anderson (sludge 1)	Marion (sludge 2)	Frankfort (sludge 3)
Carbon (%)			
Organic	15.40	7.50	10.40
Inorganic	2.03	3.70	1.80
Nitrogen, organic (%)	0.68	0.66	1.42
Phosphorus (%)	1.38	0.85	1.89
Metals (mg/kg)			
Cadmium	284	247	1,210
Nickel	2,040	215	430
Zinc	6,800	5,200	1,900
Copper	1,200	450	1,330
Lead	1,070	1,520	3,480

* Dry weight basis

Table 14.2. Test weight, protein, and ash contents of whole oats and oat fractions.

Sludge Applied	Application Rate (metric tons/ha)	Test Weight* (kg/hl)	Protein (%)*			Ash (%)*		
			Whole oats	Groats (%N x 6.25)	Hulls	Whole oats	Groats	Hulls
None	-	48.1cd	12.8ab	15.3ab	2.3abcd	3.59c	2.26bcd	6.83f
1	56	46.8cd	13.0ab	14.9ab	2.2ab	3.57abc	2.32cd	6.56ef
	112	45.6bc	13.8bc	16.1bc	2.6bcd	3.52ab	2.36cd	6.54ef
	224	44.3b	15.7de	19.3de	3.5e	3.36ab	2.01a	5.74bc
	448	39.6a	16.2e	20.1e	4.0e	3.16ab	2.37d	4.98a
2	56	47.6cd	12.4a	14.1a	1.9a	3.44ab	2.30bcd	6.35de
	112	48.2d	13.1ab	15.4ab	2.6bcd	3.42ab	2.34cd	5.99cd
	168	48.7d	14.9c	16.8c	2.1ab	3.07a	2.01a	5.51b
3	56	47.9cd	15.5c	17.0c	2.2abc	3.06a	2.22bc	5.97cd
	112	46.9cd	15.6de	19.0de	2.9d	3.21ab	2.17b	5.96cd
	168	45.8bcd	17.5d	18.4d	2.8cd	3.64bc	2.25bcd	6.34def

* For each parameter, values in a column followed by the same letter are not significantly different by Duncan's Multiple Range Test (p = .05).

Table 14.3. Concentrations of copper and nickel in whole oats and oat fractions.

Sludge Applied	Application Rate (metric tons/ha)	Applied (kg/ha)	Metal concentration (mg/kg) in*		
			Whole oats	Oat groats	Oat hulls
			Copper		
None	-	-	5.44a	5.44a	2.54a
1	56	67	6.38ab	6.16ab	4.70bcd
	112	134	8.83c	6.74abc	4.80bcde
	224	268	8.70c	8.78de	5.45bcde
	448	536	9.08c	10.40fg	5.65bcde
2	56	25	5.95a	5.72a	3.48ab
	112	50	8.20bc	7.45bc	5.88cde
	168	75	7.52abc	11.00g	6.98e
3	56	74	6.98abc	7.58cd	6.30cde
	112	148	6.10ab	6.10a	4.48abc
	168	222	9.22c	9.25ef	6.80de
			Nickel		
None	-	-	5.31ab	3.51ab	1.39a
1	56	114	10.90de	10.50c	1.88ab
	112	228	13.78e	16.45de	2.62b
	224	457	18.05f	21.75e	6.32c
	448	914	30.85g	41.80f	12.22d
2	56	12	3.92ab	1.85a	0.92a
	112	24	3.58a	2.05a	1.22a
	168	36	4.35ab	2.18a	1.28a
3	56	24	6.75bc	6.45abc	1.82ab
	112	48	9.42cd	9.00bc	2.45b
	168	72	11.90de	11.58cd	1.80ab

* For each metal, values in a column followed by the same letter are not significantly different by Duncan's Multiple Range Test (p = .05).

Table 14.4. Concentrations of zinc and cadmium in whole oats and oat fractions.

Sludge Applied	Application Rate (metric tons/ha)	Applied (kg/ha)	Metal concentration (mg/kg) in*		
			Whole oats	Oat groats	Oat hulls
			Zinc		
None	-	-	35.0a	48.8bc	31.6a
1	56	381	45.3cde	48.8bc	25.9a
	112	762	53.2ef	54.0cd	33.6a
	224	1,523	55.1f	59.0de	49.6b
	448	3,046	64.7g	64.4e	63.9b
2	56	291	30.0a	34.2a	26.8a
	112	582	35.1a	42.9b	28.8a
	168	874	36.4ab	43.2b	28.2a
3	56	106	43.7bcd	47.5bc	32.6a
	112	213	43.1bc	45.7b	31.6a
	168	320	51.5def	63.0e	27.0a
			Cadmium		
None	-	-	<0.05a	<0.05a	<0.05a
1	56	16	0.44ab	0.47b	0.19a
	112	32	0.68b	0.92c	0.28a
	224	64	1.24c	1.40d	0.99b
	448	128	2.26d	2.36e	1.57c
2	56	14	0.14a	0.20ab	<0.05a
	112	28	0.20a	0.28ab	0.10a
	168	42	0.37ab	0.50b	0.27a
3	56	68	3.86e	4.29f	1.62c
	112	136	4.68f	4.68g	1.99d
	168	203	6.61g	6.83h	3.32e

* For each metal, values in a column followed by the same letter are not significantly different by Duncan's Multiple Range Test (p = .05).

Table 14.5. Concentrations of iron and manganese in whole oats and oat fractions.

Sludge Applied	Application Rate (metric tons/ha)	Metal concentration (mg/kg) in*		
		Whole oats	Oat groats	Oat hulls
		Iron		
None	-	32.3ab	28.1a	23.2ab
1	56	35.8abc	31.8abc	38.2de
	112	48.8c	48.0def	43.7e
	224	63.8d	51.6f	40.8de
	448	64.8d	49.6ef	45.6e
2	56	45.3bc	28.9ab	28.4bc
	112	35.8abc	38.7bcd	41.1de
	168	37.7abc	36.0abc	34.6cd
3	56	37.9abc	40.2cde	16.9a
	112	28.3a	28.2a	22.9ab
	168	69.5d	69.2g	83.3f
		Manganese		
None	-	28.6cd	32.5cd	25.2d
1	56	30.3d	30.6bcd	14.6abc
	112	29.0cd	35.9d	20.6cd
	224	22.8abcd	25.2abc	12.8abc
	448	27.6bcd	24.3abc	19.7bcd
2	56	17.4a	23.3ab	10.8ab
	112	18.1a	24.6abc	8.8a
	168	29.4d	37.8d	16.9abcd
3	56	21.3abc	23.3ab	8.1a
	112	16.7a	17.5a	9.5a
	168	20.2ab	24.6abc	17.1abcd

* For each metal, values in a column followed by the same letter are not significantly different by Duncan's Multiple Range Test (p = .05).

Table 14.6. The effect of sludge application on the protein, ash and fat content of corn grain and fractions obtained by dry-milling

Sludge Applied		Concentration (%, dry weight) in*					
No.	Rate (metric tons /ha)	Whole grain	Grits	Meal	Flour	Germ	Hulls
				Protein			
None	-	6.0a	4.9a	5.5a	4.3a	15.2ab	6.6a
	-+	7.5def	6.4b	6.3b	5.2cd	15.2ab	7.9bcd
	-≠	8.0fg	6.8bc	6.8b	5.8e	15.7b	8.8e
1	56	6.5b	5.2a	5.7a	4.6ab	15.2ab	7.3b
	112	7.4de	7.1bc	6.7b	5.5de	15.3ab	7.9bcd
	448	8.4g	7.3c	7.0bc	6.4f	15.4b	9.0e
2	56	6.7bc	5.6a	5.7a	4.8bc	14.8a	7.4bc
	112	7.1cd	6.6b	6.8b	5.6de	15.6b	8.1cd
3	56	7.5def	6.6b	6.6b	5.6de	15.6b	8.2d
	112	7.7ef	7.4c	7.5c	6.6f	16.4c	9.1e
				Ash			
None	-	1.15c	0.25a	0.47d	0.41a	6.46bcd	1.10ab
	-+	1.07ab	0.28abc	0.38ab	0.42ab	5.64	1.09ab
	-≠	1.12abc	0.35de	0.37abc	0.50bc	5.43	0.95a
1	56	1.14bc	0.26a	0.46bcd	0.44ab	6.76d	1.29c
	112	1.16c	0.39e	0.48d	0.55cd	6.74d	1.32c
	448	1.16c	0.32bcd	0.36a	0.59d	6.19b	1.18bc
2	56	1.14bc	0.27ab	0.40abcd	0.38a	6.23bc	1.19ab
	112	1.08a	0.37de	0.61e	0.54cd	6.72d	1.35c
3	56	1.14bc	0.32cd	0.44abcd	0.42ab	6.16b	1.10ab
	112	1.13abc	0.38e	0.63e	0.60d	6.55cd	1.35c
				Fat			
None	-	3.7c	0.6ab	1.5d	1.2ab	23.6b	3.0abc
	-+	3.4ab	0.5a	0.8a	1.2ab	20.4a	2.7a
	-≠	3.2a	0.6ab	0.8a	1.2ab	20.6a	3.0ab
1	56	3.4ab	0.6ab	1.2bc	1.4b	23.5b	3.4c
	112	3.4ab	0.9b	1.1bc	1.1a	22.8b	2.9ab
	448	3.4ab	0.6ab	0.8a	1.7c	20.8a	3.2bc
2	56	3.4ab	0.5a	0.9ab	1.0a	20.8a	2.9ab
	112	3.3ab	0.8ab	1.4cd	1.1a	23.3b	3.1abc
3	56	3.5abc	0.9b	1.3cd	1.3ab	21.5a	2.9ab
	112	3.5abc	0.9b	1.5d	1.1a	23.6b	3.0ab

* For each parameter, values in a column followed by the same letter are not significantly different ($p = 0.05$) by Duncan's Multiple Range test.

+ Soil was fertilized with 100 kg N, 25 kg P and 50 kg K per ha.

≠ Soil was fertilized with 200 kg N, 50 kg P and 100 kg K per ha.

Table 14.7. The effect of sludge application on the cadmium, zinc and nickel content of corn grain and fractions obtained by dry-milling.

Sludge Applied		Concentration (mg/kg, dry weight) in*					
No.	Rate (metric tons /ha)	Whole grain	Grits	Meal	Flour	Germ	Hulls
				Cd			
None	-	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
	-+	<0.05	<0.05	<0.05	<0.05	0.09	0.24
	-≠	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
1	56	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
	112	<0.05	<0.05	<0.05	<0.05	<0.05	0.07
	448	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
2	56	<0.05	0.16	0.15	0.21	0.17	0.27
	112	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
3	56	<0.05	0.24	0.21	0.37	0.47	0.54
	112	<0.05	<0.05	0.05	0.09	0.41	0.25
				Ni			
None	-	0.28a	<0.20	<0.20	<0.20a	1.80b	<0.20a
	-+	0.85b	<0.20	<0.20	<0.20a	2.33b	0.79c
	-≠	<0.20a	<0.20	<0.20	<0.20a	1.46b	0.20a
1	56	1.09b	<0.20	<0.20	<0.20a	4.93d	0.20a
	112	0.88b	<0.20	0.72	0.96c	4.65d	1.73d
	448	2.30c	<0.20	<0.20	<0.20a	<0.20a	<0.20a
2	56	1.01b	<0.20	<0.20	<0.20a	2.34b	<0.20a
	112	<0.20a	0.54	0.39	0.58b	2.30b	0.88c
3	56	0.30a	<0.20	0.72	<0.20a	1.72b	0.42b
	112	0.36a	0.44	0.32	0.22a	3.52c	0.76c
				Zn			
None	-	23.6cde	4.8d	7.9def	10.6c	66a	29.5de
	-+	23.3cd	2.9ab	4.7a	6.4a	113c	20.7a
	-≠	21.1ab	4.3cd	6.8bcd	10.1c	107bc	22.7ab
1	56	25.0de	3.5bc	8.9ef	9.8bc	193d	31.8e
	112	23.5cde	7.2f	7.6de	9.2abc	104bc	26bcd
	448	28.6f	4.7d	5.8abc	17.4d	68a	28.2cd
2	56	25.3e	2.4a	5.6ab	7.2ab	102bc	22.2ab
	112	20.8ab	7.5f	8.4ef	8.0abc	95b	22.2ab
3	56	22.4bc	4.5cd	7.0cd	9.1abc	112c	22.0ab
	112	20.2a	6.0e	9.2f	9.6bc	102bc	24.5ab

* For each metal, values in a column followed by the same letter are not significantly different ($p = 0.05$) by Duncan's Multiple Range test.

+ Soil fertilized with 100 kg N, 25 kg P and 50 kg K per ha.

≠ Soil fertilized with 200 kg N, 50 kg P and 100 kg K per ha.

Table 14.8. The effect of sludge applications on the copper, iron and manganese content of corn grain and fractions obtained by dry-milling

Sludge Applied		Concentration (mg/kg, dry weight) in*					
No.	Rate (metric tons /ha)	Whole grain	Grits	Meal	Flour	Germ	Hulls
				Cu			
None	-	2.24b	1.00bcd	1.52ef	1.92cd	7.80cd	3.69e
	-+	1.88a	0.82abc	0.80abc	1.51abc	5.70ab	2.48ab
	-≠	1.95a	0.55a	0.68a	1.46abc	6.85bc	2.33ab
1	56	2.00a	0.68ab	1.09cd	1.70bc	6.08ab	2.67bc
	112	2.00a	1.06cd	1.14cde	1.83bcd	6.19ab	2.40ab
	448	2.27b	1.20e	1.29de	3.16e	21.00f	3.06cd
2	56	1.82a	0.60a	0.70ab	1.14ab	5.41a	2.07a
	112	1.82a	0.73abc	0.76abc	0.92a	8.25d	2.42ab
3	56	2.28b	1.02bcd	1.28de	1.79bcd	11.80e	3.27cd
	112	2.32b	1.42f	1.83f	2.34bcd	12.70e	3.33de
				Fe			
None	-	14.7a	4.3bc	8.0cd	19.3b	40.5ab	17.3a
	-+	18.8cd	3.6ab	5.4a	13.8a	55.8cd	18.0a
	-≠	18.0bc	6.5e	9.8ef	17.2ab	79.3e	24.7cd
1	56	16.7ab	4.8cd	10.8f	19.2b	38.7a	17.8a
	112	17.9bc	5.2cd	6.9abc	13.1a	39.0a	19.6ab
	448	19.0cd	5.0cd	6.4ab	23.3c	35.6a	25.7d
2	56	16.6ab	3.4a	5.9a	13.0a	59.5d	16.8a
	112	16.5ab	5.0c	8.6de	13.4a	39.3a	20.3abc
3	56	18.1bcd	4.8c	8.3cd	16.1a	49.8bcd	21.7abcd
	112	20.6d	5.9de	10.4f	16.6ab	45.2abc	24.0bcd
				Mn			
None	-	3.78e	0.33a	0.87de	1.18c	8.78e	3.02cd
	-+	3.59de	0.63d	0.85cde	1.36d	15.50g	4.90f
	-≠	3.71de	0.63d	0.98e	1.58e	13.60f	5.34f
1	56	3.24bc	0.60cd	0.24a	0.46a	3.76b	1.49a
	112	3.45cd	0.63d	0.66bc	1.17c	7.27de	3.52d
	448	3.26bc	0.60cd	0.79bcde	1.64e	5.21bc	4.20e
2	56	2.99b	0.54bcd	0.77bcd	1.18c	2.03a	3.46d
	112	3.27bc	0.54bcd	0.84cde	1.08cd	5.17bc	3.57d
3	56	2.20a	0.48bc	0.72bcd	0.83b	5.41bc	2.30b
	112	2.34a	0.44ab	0.62b	1.04c	5.83cd	2.79bc

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* For each metal, values in a column followed by the same letter are not significatly different (p = 0.05) by Duncan's Multiple Range test.

+ Soil fertilized with 100 kg N, 25 kg P and 50 kg K per ha.

≠ Soil fertilized with 200 kg N, 50 kg P and 100 kg K per ha.

Table 14.9. Effect of sewage sludge application on the yield, test weight, particle size index, thousand kernel weight, protein, and flour yield of wheat.*

Sludge applied							
No.	Rate (metric tons/ha)	Yield (metric tons/ha)	Test weight (kg/hl)	Thousand kernel weight (g)	Particle size index	Protein (%)	Flour yield (%)
None	-	5.26de	62.0c	41.6bc	49.1abc	13.98a	65.6a
1	56	5.59e	61.8c	41.6bc	46.6a	14.01a	65.0a
	112	4.40bcd	61.3abc	41.6bc	47.3ab	15.20b	64.4abcd
	224	3.55ab	61.0ab	40.6ab	49.1abc	16.66d	63.4bcde
	448	3.78ab	61.0ab	41.6bc	50.6bc	17.25e	62.7e
2	56	5.15de	61.8c	40.4ab	48.5abc	14.22a	64.7ab
	112	4.80cde	61.7bc	40.7ab	51.8c	15.38bc	64.5abc
	168	3.89abc	61.8c	39.8a	50.2bc	16.15d	63.0de
3	56	4.38bcd	61.6bc	42.6c	51.1c	16.09d	65.1a
	112	3.02a	60.8a	42.4c	50.3bc	17.06e	63.1cde
	168	2.93a	61.1ab	41.5bc	49.4abc	17.11e	62.7e

* Values in a column followed by the same letter are not significantly different (P = 0.05) by Duncan's Multiple Range Test.

Table 14.10. Concentrations of Zn, Cd, Cu, Ni, Fe and Mn in whole wheat, bran and flour*

Sludge applied		Concentration (mg/kg) in					
No.	Rate (metric tons/ha)	Whole wheat	Bran	Flour	Whole wheat	Bran	Flour
		Zn			Cd		
None	-	37.6a	76.8a	7.51a	0.89b	1.03ab	0.22a
1	56	49.9cd	96.2cd	10.0de	1.14bc	2.06de	0.37b
	112	51.2cd	93.0bc	10.4de	1.46c	2.42e	0.49c
	224	57.5f	112efg	10.9e	2.22d	3.37f	0.63d
	448	71.4g	143h	12.0f	2.10d	3.01f	0.75e
2	56	37.3a	86.5a	8.44ab	0.47a	0.78a	0.28ab
	112	44.6b	107ef	8.37ab	0.80ab	1.63cd	0.30ab
	168	48.1bc	103de	9.47cd	0.83ab	1.45bc	0.29ab
3	56	53.0cde	112efg	10.5de	3.44e	4.81g	1.26f
	112	54.2ef	116efg	10.2de	4.13f	5.95h	1.49g
	168	54.1def	118g	8.82bc	4.43f	6.81i	1.58g
		Cu			Ni		
None	-	4.4bc	10.0abc	0.96a	<0.10a	0.41ab	0.13b
1	56	4.7cd	10.4bc	1.29b	0.66bc	1.50cd	0.11ab
	112	5.1de	9.50abc	1.34b	0.99c	2.00d	<0.10ab
	224	5.5e	9.21ab	1.45bc	1.54d	4.00e	0.24c
	448	5.6e	12.2d	2.64g	4.47e	9.54f	0.63d
2	56	3.8a	10.9cd	1.72de	0.16a	<0.10a	<0.10ab
	112	3.8a	10.0abc	1.54c	0.24ab	0.55abc	<0.10a
	168	3.9a	9.49abc	1.55cd	<0.10a	<0.10a	<0.10a
3	56	3.9a	9.72abc	2.06f	0.14a	0.96abcd	0.26c
	112	4.0ab	9.10ab	1.86e	0.72e	1.24bcd	0.15b
	168	3.8a	8.67a	1.60cd	0.64bc	1.66d	<0.10a
		Fe			Mn		
None	-	29.8a	55.4e	8.88ab	3.96a	10.9cd	2.79c
1	56	33.7c	55.1e	8.69ab	4.64ab	11.8cd	1.71b
	112	40.0d	58.3ef	9.72abcd	5.15ab	10.2abc	1.67b
	224	43.2e	59.5f	11.2d	5.02ab	8.79ab	1.13a
	448	29.9ab	51.4d	9.82abcd	5.34ab	8.25a	1.04a
2	56	31.0ab	42.7b	8.27a	8.87c	22.0f	1.32ab
	112	32.0abc	12.2a	10.4bcd	14.3d	12.4d	1.36ab
	168	32.1bc	12.4a	9.22abc	7.51bc	11.8cd	1.05a
3	56	31.5abc	48.1cd	10.9cd	6.32abc	10.6bcd	1.09a
	112	31.1ab	47.1c	9.77abcd	6.52abc	19.2e	1.20a
	168	33.6c	13.0a	9.37abc	7.09bc	10.4bcd	1.00a

* For each parameter, values in a column followed by the same letter are not significantly different ($p = 0.05$) by Duncan's Multiple Range Test.

Table 14.11. Characteristics of flour obtained from wheat grown on soil treated with sewage sludge*

Sludge applied		Flour Protein (%)	Flour Ash (%)	Mixograph Peak Time (min.)	Cookie Diameter (cm)
No.	Rate (metric tons/ha)				
None	-	12.39a	0.50bc	3.95f	17.7f
1	56	12.68a	0.53d	3.39cde	17.6ef
	112	13.55b	0.55d	3.78ef	17.3cd
	224	15.31cd	0.54d	3.00abc	16.8a
	448	15.59d	0.52cd	2.69a	16.9a
2	56	12.55a	0.49abc	3.40cde	17.8f
	112	13.60b	0.48ab	3.11bcd	17.4def
	168	14.51c	0.47a	2.92ab	17.3cde
3	56	14.79cd	0.49abc	3.52de	17.2bcd
	112	15.66d	0.52cd	3.44de	17.0abc
	168	15.66d	0.50bc	3.31bcd	16.9ab

* Values in a column followed by the same letter are not significantly different (p = 0.05) by Duncan's Multiple Range Test.

Table 14.12. Effect of sewage sludge on the protein, fat and ash content of soybean grain and fractions*

Sludge applied		Protein (%)			Ash (%)			Fat (%)		
No.	Rate (metric tons/ha)	Whole bean	Hulls	Meal	Whole bean	Hulls	Meal	Whole bean	Hulls	Meal
None	-	37.3a	10.2d	45.3ab	4.9abc	4.4ab	6.7a	21.0abc	2.51e	0.90abcd
1	56	37.4a	9.1bc	44.5a	5.0abc	4.3a	6.6a	21.2abc	1.65cd	0.72abc
	112	37.1a	9.7c	44.8a	5.0bc	4.4ab	6.7a	21.4bcd	2.08de	0.63a
	224	36.9a	9.3bc	45.7ab	5.0bc	4.3a	6.7a	22.0d	2.32e	0.83abcd
	448	36.7a	8.5abc	45.8ab	5.0bc	4.2a	6.8a	21.9cd	1.65cd	0.99bcd
2	56	37.2a	8.4ab	45.4ab	4.9a	4.4ab	6.7a	21.1abc	1.50abc	1.13d
	112	37.1a	7.7a	45.8ab	4.9abc	4.4ab	6.8a	20.4a	0.98a	0.66ab
	168	37.4a	7.5a	46.7b	5.0bc	4.6b	6.7a	21.2abcd	1.01ab	1.03cd
3	56	39.0a	8.5abc	45.5ab	4.9ab	4.3a	6.7a	20.9ab	1.52abcd	1.00cd
	112	37.2a	8.7abc	46.0ab	4.9abc	4.3a	6.7a	21.3abcd	1.54bcd	0.89abcd
	168	37.6a	8.5abc	46.0ab	4.9abc	4.3a	6.7a	21.2abcd	1.00ab	0.62a

* Values in a column followed by the same letter are not significantly different ($p = 0.05$).

Table 14.13. Effect of sewage sludge on the Zn, Cd, Cu, Ni, Fe and Mn content of soybean grain and fractions*

Sludge applied		Metal concentration (mg/kg) in					
No.	Rate (metric tons/ha)	Whole bean	Hulls	Meal	Whole bean	Hulls	Meal
		Zn			Cd		
None	-	36a	69b	48ab	0.56ab	0.46a	0.34a
1	56	41bcd	80c	51bc	0.72abc	0.74abc	0.57abc
	112	40bc	88d	56de	0.89abc	0.81bcd	0.69c
	224	38ab	85cd	54cde	0.89abc	1.12de	0.69c
	448	50g	80c	63f	0.94c	1.28e	0.66bc
2	56	42cde	71b	53cde	0.56a	0.64ab	0.37ab
	112	44ef	69b	53cd	0.74abc	1.06cde	0.59abc
	168	45ef	67b	51bc	0.75abc	0.79bc	0.55abc
3	56	44def	86cd	47a	2.4d	3.60g	1.45d
	112	47f	73b	57e	3.3e	3.09f	1.88e
	168	47f	57a	57de	3.8f	4.17h	3.06f
		Cu			Ni		
None	-	13b	5.7cd	9.7a	5.1a	4.2a	2.4a
1	56	14bc	4.2ab	9.9a	6.8ab	4.9abc	4.2b
	112	15bc	4.0a	9.8a	7.1b	4.9abc	4.8bc
	224	13b	5.4bc	10a	7.0ab	4.7ab	4.7bc
	448	15bc	6.6d	16cd	17.9e	5.4bcd	16.9f
2	56	5.7a	4.7abc	16c	5.4ab	7.1ef	7.6d
	112	18de	4.9abc	11b	5.9ab	4.9abc	5.6c
	168	17cde	5.2abc	11b	5.3ab	5.9cd	5.3c
3	56	16bcd	4.5abc	17d	9.3c	6.1cde	12e
	112	15bc	4.9abc	17d	10.8c	6.3def	11e
	168	20e	7.9e	12b	15.9d	7.4f	7.1d
		Fe			Mn		
None	-	34b	162b	36c	17ef	6.1a	15bcd
1	56	32a	175b	35abc	14bcd	6.9ab	12abc
	112	31a	219c	35abc	14bcd	7.3abc	15bcd
	224	30a	207c	34ab	13bc	7.6abc	24e
	448	35b	70a	33a	16def	8.0bc	14bcd
2	56	35b	69a	36c	14cd	6.5ab	12abc
	112	44c	157b	36bc	20f	7.3abc	16d
	168	42c	179b	35abc	15cde	7.1ab	15cd
3	56	35b	60a	35bc	13.9cd	8.1bc	12ab
	112	35b	70a	37c	11.3cd	6.8ab	9a
	168	42c	41a	36bc	10.7a	8.8c	11ab

* For each metal, values in a column followed by the same letter are not significantly different ($p = 0.05$).

SECTION 15

ASSESSMENT OF HEAVY METAL EQUILIBRIA IN SEWAGE SLUDGE-TREATED SOIL

INTRODUCTION

The chemistry of metals in soils is complex and not completely understood. Analytical problems complicate metal studies in soils because the concentrations of the metals and ligands to be measured, particularly water-soluble metals, are often below the detection limits of conventional analytical methods (e.g., flame atomic absorption spectrophotometry). Even though techniques exist for the direct determination of metal species (e.g., polarography, chromatography, gel filtration) in solutions, the application of these procedures to soil solutions has met with limited success. However, a knowledge of metal species in the soil solution is essential to predict the reactions and plant availability of these elements in soils.

Competitive metal-chelate equilibria based on thermodynamic calculations have been used to determine metal activities in soils (Norvell, 1972; Lindsay, 1979). This approach is particularly useful when there is a lack of reliable data on metal concentrations in solution or when analytical techniques do not permit accurate assessment of the free ionic concentration and metal complexes formed with the various ligands in the system. McDuff and Morel (1974) developed the computer program REDEQL-2 to model chemical equilibria in solutions. Sposito and Mattigod (1979) subsequently developed the program GEOCHEM from REDEQL-2 by incorporating subroutines for activity coefficients at high ionic strength and for ion exchange. They also critically reviewed and expanded the data base of equilibrium constants. The program involves calculating the concentrations of all metal species based on the organic and inorganic ligands present in a soil solution. GEOCHEM is an iterative program that uses stability constants corrected for ionic strength to calculate the metal and ligand species present.

Mahler et al. (1980) used GEOCHEM to calculate the distribution of Cd species in saturation extracts of two soils amended with various levels of Cd. Free ionic Cd accounted for 67 to 73% and 64 to 68% of total Cd in saturation extracts of an acid (pH 5.1) and calcareous (pH 7.6) soil, respectively. Cadmium was primarily associated with sulfate, chloride, and fulvate ligands. More recently, Emmerich et al. (1982) found that free ionic Cd, Cu, Ni and Zn was approximately 55, 2, 65, and 70%, respectively of the total present in soil solutions from soils amended with sludge and incubated under laboratory conditions for 25 months.

The objectives of this study were to determine the concentrations of total metal ions (Zn, Cd, Cu, Ni, Pb, Mn, Fe, Ca, Mg, Al, Na, and K) in water extracts of a sludge-treated acidic soil and to calculate metal and ligand species in the soil solution.

MATERIALS AND METHODS

Experiment Design

The experiment was initiated in the fall of 1971 at the Tennessee Valley Authority Agricultural Research Farm in Muscle Shoals, Alabama, on a Sango silt loam soil (Glossic Fragiudult), with a pH of 4.9 and cation exchange capacity (CEC) of 7 cmol(NH_4^+) kg^{-1}.

Anaerobically digested, air-dried sewage sludge from Tuscumbia, Alabama was applied at rates of 50, 100, and 200 metric tons ha^{-1} and incorporated into the soil by disking to a depth of 15 cm. Soil pH after sludge application was 5.6 at the highest rate and 5.3 at the lower rates. A randomized complete block design with four replications was used in the study. Original plot dimensions were 3.6 x 15.0 m.

In the fall of 1972, all pots were subdivided into areas 3.6 x 7.5 m. One-half of each plot received additional sludge applications in 1972, 1973, and 1974 at the same rate used for the initial application (Table 15.1). The other half of each plot received no additional sludge so that residual effects from the original applications could be monitored. The plots have been cropped continuously with sweet corn (*Zea mays* L., cv. Silver Queen) since 1972.

Collection and Analysis of Soil Solutions

Soil samples were collected in the fall of 1977, air-dried, and crushed to < 2-mm. Water was added to 150-g samples to bring the soil water potential to -0.33 bars, and the samples were then incubated for 7 days at 25°C. Soil solutions were recovered by centrifugation (Gillman, 1976). The solutions were analyzed for total Cd by flameless atomic absorption spectrophotometry, using background correction. The soil solutions were also analyzed for total Zn, Mn, Fe, Al, Cu, Ni, Pb, Ca, Mg, Na, and K by flame atomic absorption or flame emission spectrophotometry; inorganic P by a molybdenum-blue method (Murphy and Riley, 1962); Cl by a Mohr titration method (Bower and Wilcox, 1965); SO_4-S by a turbidimetric method (Tabatabai, 1977); organic C by the method of Mebius (1960); and conductivity and pH by standard conductivity and pH meters, respectively. All values reported are the average of at least duplicate determinations on soil solutions obtained from 3 or 4 replicate field plots.

Calculation of Metal and Ligand Species by GEOCHEM

To predict the equilibria of a metal in solution, GEOCHEM uses iterative techniques to solve the following equations (Sposito and Mattigod, 1979) (The subscript t will be used to denote total soluble concentration):

$$M_t = M^{m+} + M\text{-}SO_4 + M\text{-}PO_4 + M\text{-}Cl + M\text{-}OH + MCO_3 + M\text{-}FA \quad [1]$$

and

$$L_t = L^{1-} + HL + ZnL + CaL + MgL + CdL + CuL + NiL + PbL + MnL + MnL + FeL + AlL + NaL + KL \quad [2]$$

Where M_t = total metal, M^{m+} = free metal with charge m+, $M\text{-}SO_4$ = metal sulfate species, $M\text{-}PO_4$ = metal phosphate species, M-Cl = metal chloride species, M-OH = metal hydroxide species, $M\text{-}CO_3$ = metal carbonate species, M-FA = metal fulvic acid species, L_t = total ligand, L^{1-} = free ligand with charge 1-, HL = sum of protonated ligand species, ZnL = sum of Zn ligand species, etc. Charges on individual species have been omitted for simplicity. Metals and ligands considered in this study were H, Cd, Zn, Cu, Ni, Pb, Mn, Fe, Al, Ca, Mg, Na, K, SO_4, PO_4, Cl, OH, CO_3, and soluble organic C (i.e., fulvates). In essence, GEOCHEM solves a series of simultaneous non-linear equations that are expressed in terms of H^+, free metal, free ligand, and appropriate equilibrium constants. Concentrations of carbonate species were calculated by assuming that the soil solution was in equilibrium with atmospheric CO_2 (0.0003 atm.).

Three approaches were employed to estimate the importance of metal interactions with soluble organic C (i.e., fulvates): 1) no organic ligands were included in the calculations; 2) a mixture model consisting of 9 organic acids with known stability constants for each metal (Sposito and Mattigod, 1979); the concentration of organic acids in the mixture model is equivalent to 226 mg C/l. The molar concentration of each organic acid was scaled to measured values of organic C in the soil solutions; 3) a fulvate model based on experimentally determined values for the conditional stability constants of sludge-derived fulvate with Ca, Cd, Cu, and Pb (Sposito et al., 1981, 1982). These authors also calculated conditional constants by correlation techniques for Mg, Mn, Fe, Zn, and Ni. It should be realized that such conditional constants are strictly valid only at pH 5 and 0.1 M ionic strength. Fortunately, the soil solutions used in this study were at pH ≃ 5.0.

RESULTS AND DISCUSSION

The amounts of sludge-borne metals applied to Sango silt loam are shown in Table 15.1. Based on current federal regulations (U.S. EPA, 1979), the maximum annual application of Cd is 2 kg ha^{-1} which corresponds to 68 t ha^{-1} of Tuscumbia sludge. For this soil, the cumulative limit on Cd application would be 10 kg ha^{-1} or 325 t ha^{-1} of Tuscumbia sludge. The cumulative amounts of Zn and Cu applied exceeded current recommendations (U.S. EPA, 1977) at 400 and 800 t ha^{-1} of sludge applied, respectively. In essence, the treatments evaluated in this study bracket the amounts of sludge-borne metals that would be applied to an acidic agricultural soil if current regulations and guidelines were used to determine application rates. However, the soils were not maintained at pH 6.5 or above as recommended for soils treated with sludges.

Concentrations of Zn, Cd, Mn, Ca, Mg, Na, K, PO_4-P, SO_4-S, and Cl in the soil solution tended to increase with sludge application, particularly in plots receiving multiple sludge applications (Table 15.2). Soil solution concentrations of Cu, Ni, Pb, and Fe were generally low (< 10^{-6} *M*) at all sludge application rates; however, soluble Cu and Ni concentrations in soil treated with sludge at 800 t ha^{-1} were markedly higher than those in soil receiving lesser amounts of sludge. Soluble Al levels in soils were not increased by sludge addition and were reduced at high loading rates probably because of the liming effect of sludge additions. Soil solution pH values and electrical conductivity levels also increased with sludge application. Application of sludge at 800 t ha^{-1} over 4 years increased the pH of the soil solution by 0.9 units and increased electrical conductivity by 2.32 dS m^{-1}. Sludge addition had a minimal effect on the level of organic C in the soil solution except for treatments receiving a total application of 200 or 800 t ha^{-1}.

Application of 200 t ha^{-1} of sludge at one time (1971) resulted in higher levels of soluble Zn, Cd, Cu, PO_4-P, and Cl, and pH values in the soil solution than a similar total amount of sludge applied in 4 consecutive annual additions of 50 t ha^{-1} yr^{-1} (Table 15.2). However, soil solutions from plots receiving a total of 200 t ha^{-1} in 4 applications had higher Mn, Al, and Na concentrations than those receiving a similar amount of sludge in a single application. Levels of Ca, Mg, K, and SO_4-S as well as the electrical conductivity of the soil solution were similar in plots receiving 200 t ha^{-1} in one or four applications. Changes in soil solution composition resulting from multiple applications as compared with a single application likely resulted from differential liming of the soil. That is, the single application of 200 t ha^{-1} in 1971 resulted in a higher soil solution pH than 4 annual applications of 50 t ha^{-1}. For all treatments, the ionic strength of the soil solutions, as calculated by GEOCHEM, was directly related to the amount of sludge applied, ranging from 7.8×10^{-4} *M* in untreated soils to 3.0×10^{-2} *M* for the 800 t ha^{-1} treatment.

The predominant species of Zn in the soil solutions was Zn^{2+} (Table 15.3). Even though sludge applications increased total soluble Zn (Zn_t) from 4×10^{-6} *M* to > 1×10^{-4} *M*, from 87 to 97% of the Zn_t was present as Zn^{2+}. For most sludge treatments, SO_4 complexes accounted for 5 to 8% of Zn_t. Zinc complexes containing PO_4, CO_3, Cl, and OH all were < 0.1% of Zn_t. Fulvate complexes (mixture model) with Zn tended to increase with sludge applied but, in all cases, < 2% of Zn_t was associated with soluble organic C. The distribution of Zn species was essentially the same after application of 200 t ha^{-1} in a single treatment or in 4 annual 50 t ha^{-1} increments. Soluble Zn_t increased to a greater extent with the single 200 t ha^{-1} sludge application (1.7×10^{-4} *M*) when compared to four increments of 50 t ha^{-1} (1.2×10^{-4} *M*).

The activity of Zn^{2+} in the soil solutions was similar to that supported by several Zn solid phases. The activity of Zn^{2+} ranged from ~ 10^{-5} to 10^{-3} *M* in solutions of pH 5 to 6 (Table 15.3). Based on stability diagrams (Lindsay, 1979), the Zn^{2+} in soil solution could be controlled by one or more of the following solids 1) $ZnFe_2O_4$ in equilibrium with soil Fe; 2) soil Zn and; 3) Zn_2SiO_4 in equilibrium with amorphous Si. At similar pH values, Zn^{2+} activities obtained from this study were greater than those previously reported for soils incubated with sewage sludge (Emmerich *et al.*, 1982). The pH range of the sludge-treated soils was too narrow to evaluate the pH dependence of Zn^{2+} as predicted by the solubility of known Zn solid phases.

The distribution of Cd species in soil solutions was nearly the same as that found for Zn. Free ionic Cd accounted for 91 to 92% of Cd_t in soil solutions of untreated soils while it ranged from 79 to 89% of Cd_t when sludge was added (Table 15.3). Sulfate complexes ranged from 2.7 to 13.0% of Cd_t and increased with sludge application. In contrast to Zn where chloro complexes were < 0.1% of Zn_t for all treatments, chloro complexes of Cd ranged from 2.9 to 7.5% of Cd_t. The percentage of Cd_t associated with fulvates (mixture model) was < 5% for all treatments and was typically < 3% of Cd_t.

The concentration of Cd_t in soil solutions markedly increased with sludge applications (Table 15.2). Soluble Cd_t was approximately 10^{-8} $\underline{M}$ in untreated soils and increased to > 10^{-6} $\underline{M}$ after 4 applications of sludge at 200 t ha^{-1}. Even with this increase in Cd_t, the percentage of Cd^{2+} remained relatively constant at 80 to 90% of Cd_t. It is also interesting to note that 4 annual applications of sludge at 50 t ha^{-1} resulted in Cd_t of 3 x 10^{-8} $\underline{M}$ whereas Cd_t was 7.9 x 10^{-7} $\underline{M}$ following a single application of sludge at 200 t ha^{-1}. Thus, Cd_t as well as Cd^{2+} will be maintained at lower levels in the soil solution by repeated annual applications of sludge as opposed to a single large addition of equal magnitude. In contrast to Zn, the solubility of Cd^{2+} was not related to any known Cd solid phases. Even though Cd^{2+} was > 10^{-6} $\underline{M}$ in some soil solutions, Cd^{2+} activities in excess of 10^{-5} $\underline{M}$ would be required to maintain equilibrium with $Cd_3(PO_4)_2$ - $FePO_4$ - soil Fe (Lindsay, 1979). The Cd^{2+} concentration was similar to the 10^{-7} $\underline{M}$ value previously used to approximate soil-borne Cd (Lindsay, 1979).

Simple correlation coefficients were calculated to evaluate the relationships between soluble and DTPA extractable Zn and Cd. Soluble Zn was significantly related to DTPA extractable Zn for both annual (r = 0.77**) (**; significant at p = 0.01) and single (r = 0.65**) applications of sludge (Fig. 15.1). Correlation coefficients for soluble and DTPA extractable Cd were 0.90** and 0.93** for single and multiple sludge additions, respectively (Fig. 15.2). The Cd, and to lesser extent, Zn data indicate that a lower soluble metal concentration will be maintained by repeated annual applications of sludge than by a single addition at an equivalent cumulative rate. Concurrently, DTPA extractable levels are increased to a similar extent by either repeated or single additions of sludge to soils.

The speciation of Mn, Cu, and Ni in soil solutions is shown in Table 15.4. For Mn, the majority of soil solutions contained from 95 to 98% of the Mn_t as Mn^{2+} (Table 15.4). Sulfate complexes were present in systems but constituted only 2 to 8% of Mn_t. Manganese complexes with fulvates (mixture model) constituted from 9.5 to 17.9% of Mn_t in soils treated with sludge at 200 to 800 t ha^{-1}. Data are presented for only the 200 and 800 t ha^{-1} treatments for Cu and the 800 t ha^{-1} treatment for Ni, because most solutions contained nondetectable concentrations of these metals (detection limits for Cu and Ni were 1.7 x 10^{-6} $\underline{M}$ and 1.8 x 10^{-6} $\underline{M}$, respectively). Thus, the results obtained do not allow evaluation of the effect of sludge additions on metal and ligand speciation. A significant percentage of Cu was present as fulvate complexes (mixture model) (~ 80% of Cu_t). The amount of Ni associated with fulvates (mixture model) was similar to that for Zn and Cd. The only other Cu and Ni species present were SO_4 complexes of Cu and the SO_4 and PO_4 complexes of Ni. The limited amount of data for soluble Cu and Ni precludes any speculation about which solid phases control solubility.

The interpretation of metal complexation by soluble organic C in soil solutions must be viewed with caution because of the uncertainty associated with stability constants for fulvate-metal interactions. To facilitate interpretation of the data obtained, GEOCHEM calculations were performed using 3 sets of conditions: 1) metal-organic complexes were ignored; 2) metal-organic complexes were approximated by a mixture model (Mattigod and Sposito, 1979) and; 3) metal-organic complexes were modeled using stability constants obtained experimentally (Sposito et al., 1981) for a sludge-derived fulvic acid (fulvate model). The mixture model predicted that < 16% of the Cd_t, Zn_t and Mn_t in solution was complexed with organic and inorganic ligands (Table 15.3 and 15.4) and thus, it was not surprising that exclusion of the mixture model from GEOCHEM had a minor effect on the speciation of Cd, Zn and Mn (Table 15.5). Inorganic ligands (SO_4, PO_4 and Cl) typically accounted for > 95% of the metal complexes present. Valid calculations for Cu were performed for only the single and multiple applications at 200 t ha^{-1}. The percent Cu^{2+} increased from 19 to ~ 90% of Cu_t when the mixture model was not included in the calculations. This strong degree of Cu complexation by fulvates is consistent with previous research on the forms of soluble Cu (Hodgson et al., 1965).

The fulvate model predicted smaller free metal concentrations of Cd, Zn and Mn than the mixture model: the percent free metal was decreased by 3 to 29% by using the fulvate rather than mixture model (Table 15.5). In contrast, the fulvate and mixture models predicted that Cu^{2+} would be 69 and 19%, respectively, of Cu_t. A similar result has been reported for GEOCHEM calculations performed on data from saturation extracts of soils treated with Cd-enriched sewage sludge (Sposito et al., 1982). Stability constants for Cu with the organic ligands used in the mixture model are considerably greater than those for Cd, Zn and Mn whereas all metals (Cu, Cd, Zn and Mn) possess comparable stability constants with ligands in the fulvate model. In the fulvate model, the metals associated with the organic ligands will be proportional to the total metal concentrations present because the conditional stability constants are similar for all metal-ligand complexes. In general, the fulvate and mixture models for metal complexation by soluble organic C in soil solutions yielded similar qualitative results for the speciation of Zn, Cd, Mn and Ni. As expected, metal complexation by soluble organic C tended to increase with increasing organic C. The percentages of free Cd^{2+}, Zn^{2+}, and Mn^{2+} present in soil solutions likely range from 65 to 95% of the total present.

In summary, in excess of 80% of the Cd, Zn, Ni and Mn in an acid soil amended with sewage sludge was likely present as the free metal ion. Inorganic complexes typically comprised < 5% of the soluble metals. Since the soil solutions were in the pH 5 to 6 range, it is possible that liming the soil would result in greater complexation of metals by soluble organic C. Some discrepancies were observed when the degree of metal complexation by fulvates was computed by GEOCHEM using one model based on experimental data for sludge-derived fulvates and another based on a mixture of organic acids. The mixture model yielded greater estimates of Cu complexation by fulvates while estimates for Mn were lower. It must be noted that the fulvate model is strictly valid only at pH 5 and an ionic strength of 0.1 M. Irrespective of the approach chosen to model metal reactions with soluble organic C, the GEOCHEM calculations indicate that Zn^{2+} and Cd^{2+} are the predominant species present in the soil solution of acid soils treated with sludges at metal additions approaching the maximum recommendations for growth of agronomic crops.

Table 15.1. Metal loading rates for plots amended with Tuscumbia sewage sludge

Metal applied	Sludge applied, t ha^{-1} *							
	A				B			
	0	50	100	200	0	200	400	800
	kg ha^{-1}							
Zn	0	90	180	360	0	360	720	1,440
Cd	0	1.5	3	6	0	6	12	24
Cu	0	26	52	104	0	104	208	416
Ni	0	2	4	8	0	8	16	32
Pb	0	80	160	320	0	320	640	1,280
Mn	0	10	20	40	0	40	80	160

* A -- a single application in 1971; B -- repeat applications of initial rate in 1972, 1973, and 1974

Table 15.2. Chemical composition of soil solutions from untreated and sludge-treated soils.

Parameter	Units	Sludge applied, t ha^{-1} *							
		A				B			
		0	50	100	200	0	200	400	800
Zn	x 10^{-5} M	0.35	7.94	10.72	16.98	0.40	11.75	16.60	36.31
Cd	x 10^{-8} M	0.98	2.24	3.09	79.4	1.26	3.02	58.9	144.5
Cu	x 10^{-6} M	<1.0	<1.0	<1.0	3.16	<1.0	<1.0	<1.0	6.31
Ni	x 10^{-6} M	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	6.76
Pb	x 10^{-6} M	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Mn	x 10^{-4} M	3.72	3.09	3.89	3.55	3.63	5.37	6.46	7.41
Fe	x 10^{-6} M	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Al	x 10^{-4} M	1.86	1.86	1.86	<1.0	1.86	1.86	<1.0	<1.0
Ca	x 10^{-3} M	1.95	3.63	3.72	4.07	2.19	3.89	4.68	9.12
Mg	x 10^{-4} M	1.20	5.89	4.07	4.37	0.95	4.37	4.68	6.31
K	x 10^{-4} M	7.59	19.95	10.23	9.55	3.13	10.0	10.72	11.75
Na	x 10^{-3} M	3.31	3.89	3.89	3.98	2.51	8.32	8.51	9.12
PO_4-P	x 10^{-6} M	2.6	2.9	4.2	15.49	2.57	9.33	26.92	60.26
SO_4-S	x 10^{-3} M	0.26	0.85	0.98	1.15	0.44	0.91	1.66	3.39
Cl^-	x 10^{-3} M	0.60	1.55	1.29	1.23	0.51	0.72	1.07	1.35
Organic C g m^{-3}		160	130	160	190	170	150	180	250
Conductivity dS m^{-1}		1.54	2.25	2.14	2.08	1.56	2.03	2.38	3.88
pH		4.7	4.9	5.1	5.6	4.6	5.0	5.2	5.5

* A--a single application in 1971; B--repeat applications of initial rate in 1972, 1973, and 1974.

Table 15.3. Concentration and speciation of Zn and Cd in the soil solution as affected by sludge rate and frequency of application

Sludge applied	No. of applic.*	Total Zn	Free Zn^{2+}	Complexed Zn†		Total Cd	Free Cd^{2+}	Complexed Cd†		
				SO_4	MM‡			SO_4	Cl	MM‡
$t\ ha^{-1}y^{-1}$		x 10^{-5} M	---- % of total ----			x 10^{-8} M	-------- % of total -------			
0	0	0.35	97	2.2	0.5	0.98	92	2.7	3.6	1.7
	0	0.40	96	3.5	0.4	1.3	91	4.2	2.9	1.5
50	1	7.9	94	5.3	0.4	2.2	85	6.0	7.5	1.5
	4	4.7	94	5.0	0.7	3.0	89	5.9	3.3	1.9
100	1	10.7	93	6.0	0.9	3.1	84	6.9	6.2	2.4
	4	16.6	91	8.1	1.0	59	83	9.4	4.4	2.8
200	1	17.0	91	6.7	1.7	79	82	7.7	5.6	4.6
	4	36.3	87	11.3	1.3	144	79	13.0	4.6	3.6

* A single application in 1971; repeat applications of initial rate in 1972, 1973, and 1974.

† Complexes not given represented < 0.1% of total soluble metal.

‡ Mixture model for fulvate (Mattigod and Sposito, 1979).

Table 15.4. Concentration and speciation of Mn, Cu and Ni in the soil solution as affected by rate and frequency of sludge application

Metal	Sludge applied	No. of applic.*	Total concentration	Free metal	Metal complexes		
					SO_4	PO_4	MM†
	t $ha^{-1}y^{-1}$		x 10^{-5} M	----------- % of total metal -------------			
Mn	0	0	37	98	1.8	<0.1	0.3
		0	36	97	2.8	<0.1	0.3
	50	1	31	95	4.2	<0.1	0.3
		4	54	95	4.0	<0.1	0.4
	100	1	39	95	4.9	<0.1	0.5
		4	65	84	6.0	<0.1	9.5
	200	1	36	77	4.5	<0.1	17.9
		4	74	80	8.3	0.2	11.4
Cu	200	1	0.32	19	1.4	<0.1	79.8
	200	4	0.63	19	2.5	<0.1	78.3
Ni	200	4	0.68	87	9.0	0.1	3.3

* Single application in 1971; repeat applications of initial rate in 1972, 1973, and 1974.
† Mixture model for fulvate (Mattigod and Sposito, 1979).

Table 15.5. Effect of excluding organic ligands from consideration by GEOCHEM on the percentage of free metals in soil solution

Sludge applied*		Organic ligands included†	Cd		Zn		Cu		Ni		Mn	
A	B		A	B	A	B	A	B	A	B	A	B
--- t ha^{-1} ----			----- Calculated % of total present as free metal ion ------									
0	0	Fulvate model	67	67	79	80	--‡	--	--	--	69	73
		Mixture model	92	91	97	96	--	--	--	--	98	97
		None	94	93	98	96	--	--	--	--	98	97
50	200	Fulvate model	69	72	84	84	--	--	--	--	80	81
		Mixture model	85	89	94	94	--	--	--	--	95	95
		None	86	90	95	95	--	--	--	--	96	96
100	400	Fulvate model	66	67	80	81	--	--	--	--	74	78
		Mixture model	84	83	93	91	--	--	--	--	95	84
		None	86	86	94	92	--	--	--	--	95	93
200	800	Fulvate model	64	63	79	78	69	69	--	72	74	76
		Mixture model	82	79	91	87	19	19	--	87	77	80
		None	86	82	93	88	93	88	--	90	94	90

* A--1 application in 1971; B--repeat applications of initial rate in 1972, 1973, and 1974.

† Fulvate model - Calculations based on equilibrium constants for fulvic acid extracted from sewage sludge (Sposito *et al*., 1981); Mixture model - mixture model for soluble fulvic acid (Mattigod and Sposito, 1979).

‡ Total concentrations below detection limits of analytical methods used.

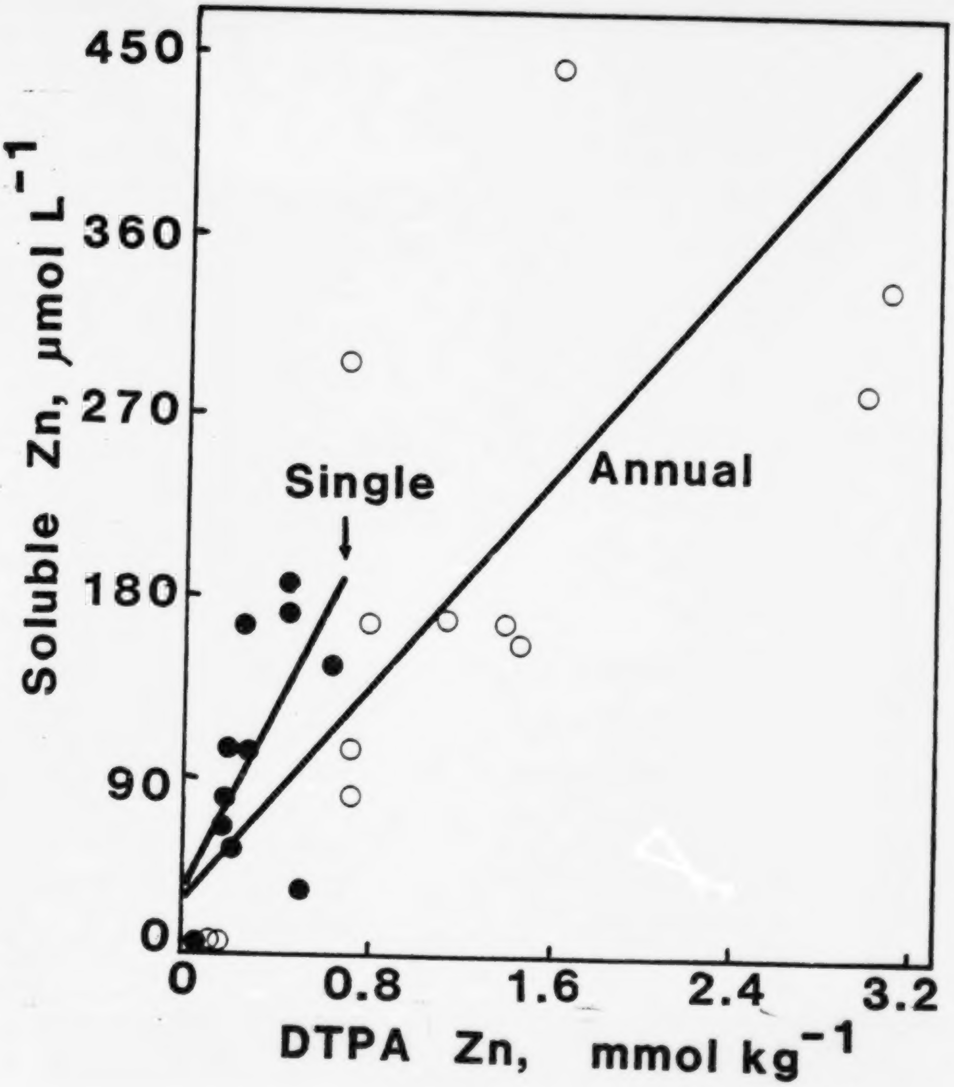

Figure 15.1. Relationship between DTPA-extractable zinc and the zinc concentration in soil solution from plots receiving single or multiple applications of sludge. Regression equations are:

Annual:	$y = 0.14x + 2.0$	$r = 0.77^{**}$
Single:	$y = 0.25x + 1.5$	$r = 0.65^{**}$

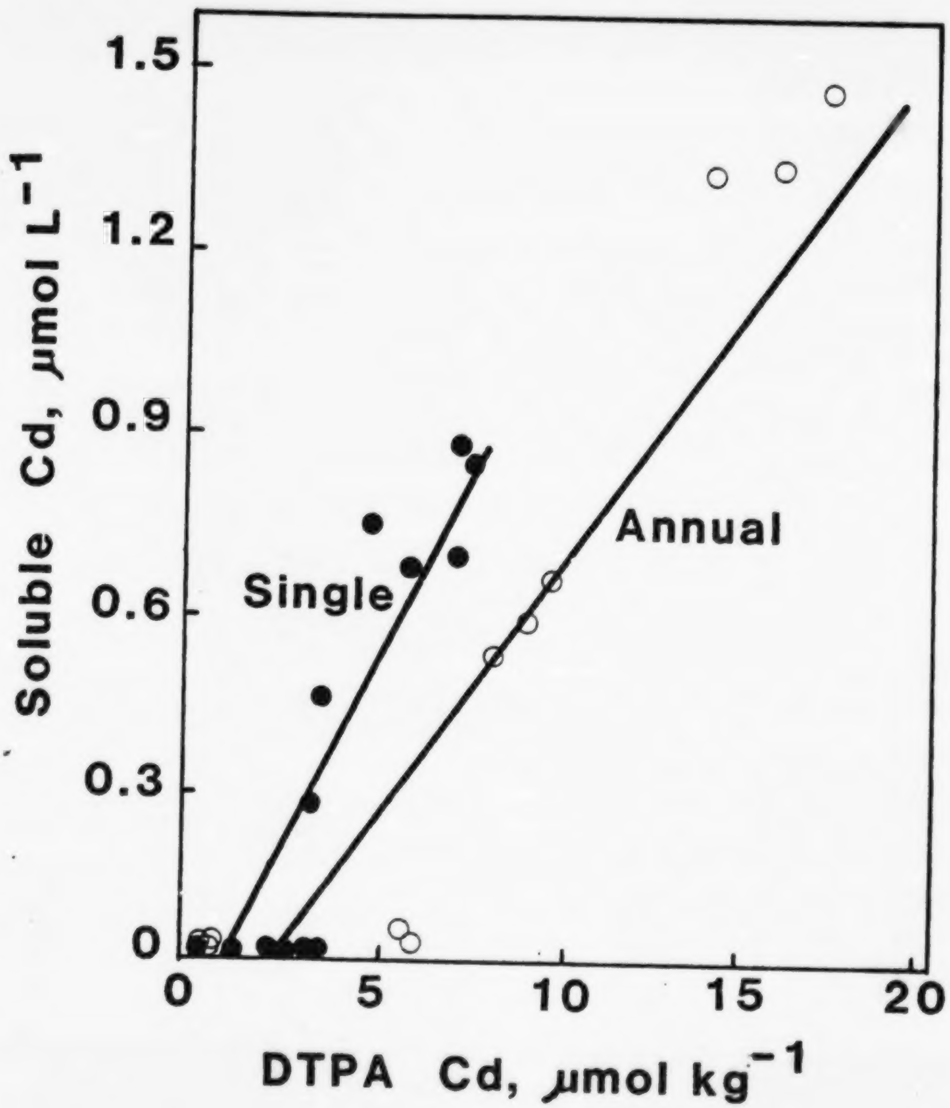

Figure 15.2. Relationship between DTPA-extractable cadmium and the cadmium concentration in the soil solution from plots receiving single or multiple applications of sludge. Regression equations are:

Annual: $y = 97.4x - 23.0$ $r = 0.93^{**}$

Single: $y = 147.8x - 20.3$ $r = 0.90^{**}$

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